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# PLANT AND SOIL

## International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

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MARTINUS NIJHOFF THE HAGUE

# PLANT AND SOIL

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# EFFECT OF INOCULUM SIZE AND NUTRITION ON THE HOST/PARASITE RELATIONS OF *STRIGA SENEGALENSIS* ON SORGHUM

by C. N. WILLIAMS *

University College, Ibadan, Nigeria

## INTRODUCTION

In the literature there is little quantitative information on the effect of nutritional factors on the relationships of the root parasites *Striga* spp. to their hosts. In field trials, Pearson [5] reports the application of sodium nitrate at a rate of 150 pounds per acre followed by 100 pounds of common salt slightly reduced infection by *Striga asiatica* Benth. (= *S. lutea* Lour.), and two successive applications of sodium nitrate also reduced infection. F. T. Last (personal communication) working with *Striga hermonthica* (Del.) Benth. on Sorghum, in the Sudan found that field applications of nitrogen (ammonium sulphata or urea) in amounts up to 80 pounds per acre, usually decreased the incidence of the parasite, but on very infertile and heavily infested soil, nitrogen increased the amounts of the parasite. Using sand cultures of *Andropogon sorghum* Hack, innoculated with *S. asiatica* and *S. densiflora* Benth., Solomon [7] found that increasing the concentration of a balanced nutrient medium, progressively reduced the development of the parasites and reduced the loss caused to the host, and this could be correlated with increased osmotic pressure of the host sap.

This paper reports the results of greenhouse experiments with *S. senegalensis* Benth. on Sorghum. Using sand cultures, the effects on host/parasite relations of 1) inoculum size, 2) deficiency of N, P and K, 3) high and low levels of a balanced nutrient medium and 4) high levels of various single salt nutrients, have been studied.

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## METHODS

Plants were grown in coarse, washed river sand in 8-inch unglazed pots. Generally four host plants (*Sorghum vulgare* var. Light Mori from Nigeria) were grown in each pot but in one experiment eight plants per pot were used. The hosts were germinated one day prior to planting. Three times as many hosts as finally required were planted in each pot and these were thinned to give an even stand two days after planting. Parasite seeds were mixed into the top 4″ of sand in the pots. In most of the experiments one gram of seed material consisting of finely ground seed pods containing about 1% (vol.) of viable Striga seeds (several hundred seeds) was inoculated into each pot. In two treatments the inoculum size was increased by thirty, by using 0.3 g of a pure sample of seeds from the same area.

The basic nutrient medium used in the cultures had the following composition as anhydrous salts: $Ca(NO_3)_2$ 10 g, $KNO_3$ 5 g, $KH_2PO_4$ 2.5 g, $MgSO_4$ 2.5 g, NaCl 0.25 g, boric acid 12.5 mg, and $FeCl_3$ 100 mg, water – to make – 1000 ml, (Medium A). In the deficiency experiments, nitrates were replaced by chlorides, phosphate by chloride, and potassium by sodium. To each of the deficient media 1/15 by volume of the full nutrient solution was added. To test the effect of increasing the concentration of the balanced medium. the level of nutrients in medium A was increased by four. To study the effects of high level of various single salts, medium A was supplemented with respectively, *M*/5 $Ca(NO_3)_2$, *M*/5 $KH_2PO_4$, *M*/5 NaCl and *M*/5 Mg $SO_4$. All media were adjusted to pH 4 with HCl before use, the Fe being added after pH adjustment. Feeding was carried out by applying 10 cc of nutrient medium to each pot thrice weekly. The sand in the pots was kept moist by adding water twice daily and each week the stale nutrient solutions were washed through with excess water. Ten pots were used for each treatment, the effects of the parasite being assessed by comparison with controls.

## EXPERIMENTAL RESULTS

### *The development of parasitism under heavy and light inoculum*

Briefly, it may be stated that *Striga senegalensis* becomes attached to the host roots shortly after germination and grows for a period of time as a total parasite beneath the soil surface. After emergence green leaves develop which photosynthesise and the plant becomes a semi-parasite.

Figure 1A shows the variation in growth rate (height) with time, of hosts inoculated with *S. senegalensis* at a rate of one gram of seed mixture containing *ca* 1 per cent viable seed, and growth rate variation of a control series without parasites. Four plants were grown in each pot and these received a balanced nutrient medium A, as above, the figures adjacent the curve indicate the numbers of

parasites showing. It is apparent that under these conditions, reduction in growth of the host occurred only after the appearance of the parasites above the surface of the sand; before this the infected host grew faster. This pattern was repeated in all of the nutritional experiments recorded below. These results were surprising in view of the often encountered statement that *Striga* spp. cause the greatest loss to the host as a total parasite beneath the soil, yet in the present series of experiments loss to the host was considerable after emergence (70.7 per cent in the series shown in Fig. 1A), while before emergence, only stimulation occurred.

In view of these results two further experiments were carried out in which the inoculum size was increased by thirty. The results

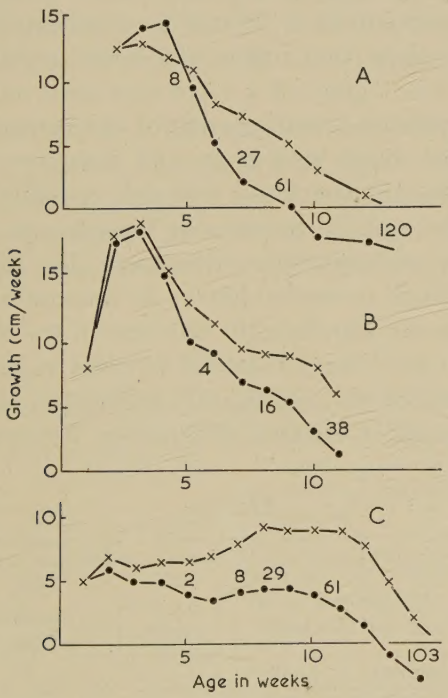


Fig. 1. Variations in growth rate with time of *Sorghum vulgare* plants, •—• infected with *Striga senegalensis*, ×—× controls.

A—pots inoculated with 1 g of a 1% Striga seed mixture with 4 host plants per pot; B—pots inoculated with 0.3 g of pure Striga seed and with 4 hosts per pot; C— as for B, but with 8 hosts plants per pot.

The numbers adjacent the curves represent the numbers of parasites emerged.

of growth rate measurements are shown in Figure 1B and 1C. In Fig. 1B, four plants were grown in each pot, and a higher rate of feeding was used (medium A × 4) since the greatest pre-emergence stimulation occurred at this level with the lower inoculum. In Fig. 1C, medium A was used for feeding but eight plants were grown in each pot. It can be seen that with the heavier inoculum, host loss commences before parasite emergence, but is greatest after emergence. In 1B, the number of parasites emerging was significantly lower than in the corresponding treatment with the low inoculum.

*Effect of deficiency in N, P and K on parasite/host relations*

In this experiment four feeding treatments were used: feeding with medium A (designated all nutrients in Table 1) and feeding with solutions deficient in N, P and K as indicated above. In this experiment and those that follow, the lower inoculum of parasite seed was used (*i.e.* 1 gram of a 1 per cent seed mixture). The experiment was continued until growth of the control plants ceased (13 weeks), after which both hosts and parasites were harvested and dried to constant weight. It was only possible to separate the emerged parasites and the major root branches, as the finer branches presented a compact interwoven mass. The results are shown in Table 1. Although somewhat lower, the amount of loss caused by the parasite was not significantly different in the deficiency treatments from that receiving a balanced nutrient supply. The number of emerged parasites was significantly reduced in the —N and —P treatments. The most striking differences, however, are seen in

TABLE 1

Effect of deficiency in N, P, and K on parasite/host relations

| Treatment | Average host wt. (g) per pot (dry basis) | | Av. parasite wt. (g) per pot dry | Loss caused by parasite % * | No. parasites emerged (10 pots) ** | Para host (wt/ |
|---|---|---|---|---|---|---|
| | Infected | Control | | | | |
| All nutrients | 6.60 | 22.5 | 1.24 | 70.7 | 120 | 0. |
| –N | 0.91 | 2.04 | 0.60 | 55.4 | 34 | 0. |
| –P | 2.09 | 4.96 | 0.96 | 57.9 | 42 | 0. |
| –K | 4.34 | 10.9 | 1.50 | 60.2 | 72 | 0. |

* $P < 0.05$ for a difference of 54% between treatments.
** $P < 0.05$ ,, ,, ,, ,, 65 parasites between treatments.
† $P < 0.05$ ,, ,, ,, ,, 0.08 between treatment.

the parasite/host ratios, these being much higher in the deficiency treatments. It thus appears that deficient hosts are able to support a greater relative weight of parasites without undergoing increased loss.

*Effect of a high level of a balanced nutrient medium and high levels of single salt nutrients.*

A comparison was made of host/parasite relations of cultures receiving an adequate supply of mineral nutrients for normal growth (medium A) (all nutrients, low concentration in Table 2), and cultures receiving 1. a fourfold increase in the level of nutrient supply and 2. medium A. supplemented with respectively, high levels of $Ca(NO_3)_2$, $KH_2PO_4$, NaCl, and Mg $SO_4$. This experiment was carried out simultaneously with the deficiency experiment above, the plants again being harvested after 13 weeks. The results are shown in Table 2.

Increasing the level of a balanced nutrient medium resulted in only a small increase in the growth of the control plants (22.5 against 26.8 g) indicating that the lower level of nutrient supply was almost adequate for normal growth (the addition of a high level of $Ca(NO_3)_2$ did, however, result in a marked increase in yield which

TABLE 2

Effects of a high level of a balanced nutrient medium and high levels of single salt nutrients.

| Treatment | Average host wt. (g) per pot (dry basis) | | Average Striga wt. (g) per pot (dry basis) | Loss caused by parasite * % | No parasites emerged ** (10 pots) | Parasite/ host ratio (wt./wt.) |
|---|---|---|---|---|---|---|
| | Infected | Control | | | | |
| trients (low conc.) | 6.6 | 22.5 | 1.24 | 70.7 | 120 | 0.19 |
| utrients + *M*/5 $Ca(NO_3)_2$ | 32.5 | 44.5 | 0.72 | 26.9 | 61 | 0.02 |
| utrients + *M*/5 $KH_2PO_4$ | 7.3 | 23.7 | 2.16 | 69.0 | 98 | 0.30 |
| utrients + *M*/5 NaCl | 7.3 | 18.1 | 1.29 | 59.7 | 72 | 0.18 |
| utrients + *M*/5 $MgSO_4$ | 7.3 | 18.0 | 1.66 | 59.4 | 88 | 0.23 |
| tr ents (high conc.) | 25.4 | 26.8 | 1.26 | 5.2 | 104 | 0.05 |

< 0.05 for a difference of 54% between treatments.
< 0.05 ,, ,, ,, ,, 65 parasites between treatments

may be taken to indicate a deficiency of Ca or N, on the other hand, excessive nitrogen in relation to other nutrients, particularly K, is well known to encourage the rank growth of plants and this may be accompanied by a reduced development of strengthening tissues *etc.*). With parasitised plants, the higher level of the balanced medium resulted in a four-fold increase in growth and a reduction of the loss caused by the parasite from 70.7 to 5.2 per cent. It can also be seen that this gain was not brought about by reduction of parasite growth, there being no significant difference in the number or weight of parasites emerging. At the lower level of nutrients the parasite/host ratio is higher but this is accompanied by increased loss, unlike the deficiency treatments above. It is clear that the host has been able to overcome the reducing effects of the parasite without reduction to the parasite, presumably by producing new roots to replace the infected ones.

Of the various single salt nutrients, $Ca(NO_3)_2$ had the most striking effect. There was a marked reduction in the loss caused by the parasite, but unlike the high level of balanced medium, there was a significant reduction in the number and weight of parasites emerged. It would appear that an unbalanced level of this salt is detrimental to the development of the parasite. In spite of increased growth of the host, however, this treatment was not as effective in overcoming the loss caused by the parasite, as a high level of balanced medium, although the absolute weight of the infected host was higher on this medium than on the balanced high level medium.

Potassium phosphate did not reduce the effect of the parasite on the contrary this produced an increase in the weight of parasite emerged. Parasites also appeared earlier than in the other treatments. Magnesium sulphate also gave a slight increase in the weight of parasite emerged and was slightly inhibitory to host growth at the level used. Sodium chloride was inhibitory to host growth and produced significantly fewer parasites. The parasite weight, however, was not significantly altered by sodium chloride.

## DISCUSSION

The action of root parasites of this type, in bringing about reduction in host growth has been much debated. One of the notable features of the parasitism of these plants, is the low efficiency of

the process, a large host reduction generally being caused for a comparatively small amount of parasite growth. Uttaman [9] tested extracts of *S. asiatica* on rice seedlings and reported wilting and death of the seedlings. This supports the often stated view that the parasite produces a toxin which results in reduction in growth of the host. With *S. senegalensis*, however, this does not appear to be the case as the data recorded in Fig. 1A shows that considerable loss to the host can occur under conditions which cause no loss before the parasite emerges above the soil surface. The beginning of host reduction coincides with the opening up of the parasite transpiration stream. This points strongly to hydroparasitism as one of the major causes of loss to the host, and would account for the low efficiency of the process. In this connection, Saunders [6], states that the *S. asiatica* reduces the transpiration of the host plants, especially during the warmer part of the day.

The results of the mineral deficiency experiments reported above (Table 1) support the view that hydroparasitism is probably a major cause of loss of the host, at least under the conditions of growth in pot cultures. It was seen that deficiency in N, P, and K did not significantly affect the loss caused by the parasite. The parasite/host ratio, however, was much higher with the deficient hosts; in other words, the deficient hosts were able to support a greater relative weight of parasite without undergoing additional loss. This would be expected if the main reduction was brought about by a drought effect, since the water requirements of the slow-growing deficient plants would not be as great as the fast-growing plants on a full nutrient medium; factors other than water being limiting. In this connection, it has often been observed that the wild grasses acting as natural hosts to species of *Striga* do not show the marked reduction characteristic of crop plants, and this might be explained by the generally slower growth rate and more rigid water economy of the wild hosts, compared with rapidly growing crop plants such as maize or sorghum.

Figure 1A shows that the growth of infected hosts was at first faster than the controls in this treatment. Stimulation also occurred in the other mineral treatments. The reason for this stimulation is obscure. It is possible that the parasite produces a factor stimulatory to the host in the first instance, on the other hand some factor may have been introduced with the dead material of the

inoculum since this consisted of ground seed pods containing about 1% of viable seed; *e.g.* a micro-nutrient which may have been limiting the growth of the controls.

Although loss to the host occurs only after emergence of the parasites with a comparatively light inoculum, a very much heavier inoculum resulted in pre-emergence loss to the host (Fig. 1B and C). In very severe infections with *S. asiatica*, the parasites may not reach the surface of the soil at all (Saunders [6]). Similarly, in the treatment illustrated in Fig. 1B, the number of emerged parasites was significantly less than in the corresponding treatment with the lower inoculum.

While beneath the surface of the soil these parasites depend entirely on the host for elaborated material, after emergence they become green and photosynthesise in the normal way. Uttaman [8] has shown that *S. asiatica* contacts the phloem regions of the host root while underground, but contacts made after emergence are with the xylem regions of the host root. *S. senegalensis* apparently continues to take elaborated material after emergence. In my experiments occasional albino parasites were produced which continued to grow for some weeks. In an attempt to determine the extent of parasitism after emergence, parasites were covered with light-proof cardboard sleeves immediately they appeared above the soil. Ventilation was provided through a light trap at the top of each sleeve. As controls, others were covered with similar transparent celluloid sleeves. Altogether the growth of 15 dark-treated and 15 light-treated parasites was recorded. As seen in Figure 2 the dark parasites grew less rapidly than the light ones over most of the period, growth rate decreasing steadily as the parasites increased in size.

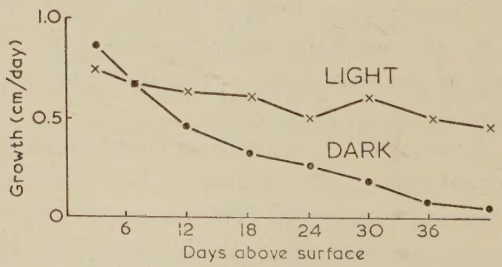


Fig. 2. Variations in growth rate with time, of *Striga senegalensis* plants, ×—× plants grown in light, •—• plants grown in the dark.

The initial growth of light parasites, however, was slower. The initial more rapid growth of the dark parasites is reminiscent of etiolation in normal plants. Growth of the dark parasites was more variable after the first week than the controls, which probably reflects the amount of connection with phloem regions of the host root. A few plants were still growing actively at the end of the experimental period while others had stopped entirely. In this experiment none of the dark parasites reached flowering as the size of the light-proof sleeves prevented continuation of the experiment. A. A. Nelson, however, (personal communication) working in America, has grown *S. asiatica* to flowering completely in the dark.

The reduction of parasite number and host loss by high levels of $Ca(NO_3)_2$ is of interest. Comparison with Pearsons' [5] experiments with *S. asiatica* in which sodium nitrate reduced infection, and those of F. T. Last with *S. hermonthica* in which field dressings of ammonium sulphate of urea reduced infection and increased yield, suggest that the nitrogen fraction of the $Ca(NO_3)_2$ is probably the effective part of the molecule. It is of interest to compare the three treatments —N, full solution (medium A), and medium A + *M*/5 $CaNO_3$ in Tables 1 and 2. In these three treatments the level of N is progressively increased. It can be seen that, in terms of numbers of parasites, an increase of nitrogen from low (—N) to adequate (medium A) results in an increase in parasite number from 34 to 120 per treatment (parasite weight is also significantly increased); a further increase in nitrogen, however, resulted in a reduction to 61 parasites, and a significant reduction in parasite weight. These results are similar to those of F. T. Last with *S. hermonthica* in which field application of N, on severely infested very infertile land at first increased the number of parasite stems while higher applications decreased them. It is evident that a moderate amount of nitrogen favours the parasite, with both *S. hermonthica* and *S. senegalensis*. This may be related to more extensive root growth of the host with moderate nitrogen, since Andrews [1] has shown that the number of parasites (*S. hermonthica*) is directly related to the extent of the host-root system. It is obvious that these facts should be considered in fertiliser treatments designed to reduce the incidence of parasitism. On the other hand, high levels of nitrogen are likely to be effective in reducing the parasite. The mechanism of parasite reduction as a consequence of applying $Ca(NO_3)_2$ is un-

known, although this is at present under investigation. Since the number of parasites was reduced it is possible that germination of the seed is affected, although with *S. hermonthica* growth of the parasite also appears to be reduced. Last observed that *S. hermonthica* plants were stunted and produced few flowers with N applications. This condition he compares to that of parasites grown on legumes (*e.g. Dolichos lablab* L., *Arachis hypogea* L. and *Vigna unguiculata* Walp.), plants high in nitrogen. On these, the parasites are generally stunted and rarely produce flowers (Andrews [2]). Last compares the effect of N on Striga to that on the host/parasite balance between cereals and the fungus parasite, *Ophiobolus graminis* Sac., the severity of this fungus parasite decreasing with increasing amounts of N (Glynne [4]). Growth of individual parasites was not measured in my experiments, although it would seem from the numbers and total weight of parasites (Table 2) that growth was not radically altered at this level of calcium nitrate. Possibly increasing the level yet further would result in reduced growth of individual parasites.

A comparison of the effect of high $Ca(NO_3)_2$ and a high level of a balanced nutrient medium (Table 2) shows that, while with the former treatment the number of parasites is reduced, with the latter, host loss is reduced but the parasite growth is unaltered. It is possible that the other constituents of the balanced medium reduced the deleterious effect of $Ca(NO_3)_2$ on the parasite. It can be seen from Table 2 that high levels of $KH_2PO_4$ and also to a lesser extent $MgSO_4$, favoured the growth of the parasite (on a weight basis), although the parasite number was not significantly different. With *S. asiatica*, however, increasing the level of a balanced nutrient medium progressively reduced the number and weight of parasites (Solomon [7]). It is possible that a further increase in the concentration of the balanced medium may also reduce *S. senegalensis*.

From Table 2 it can be seen that the high balanced medium was more effective in overcoming the loss to the host than the high calcium nitrate medium, in spite of increased shoot growth and reduced parasite number in the latter. Possibly better root growth occurred with the balanced medium, enabling the host to open up new pathways for the transport of nutrients to the shoots. There is evidence that high nitrate reduces root growth by affecting auxin metabolism (Bosemark [3]). On the other hand the increased growth

with high calcium nitrate, could lead to a lowered efficiency of the parasitic process if the water requirements of the host was simultaneously increased.

## SUMMARY

1. Using sand cultures, studies were made on the effects of inoculum size, and varying conditions of mineral nutrition on the host/parasite relations of *Striga senegalensis* on *Sorghum vulgare*.

2. The parasite grows for a period beneath the soil surface as a total parasite on the host roots after which it emerges, turns green, and becomes a semi-parasite.

3. With a low inoculum of parasite seed (1 g of a 1 per cent seed mixture) the loss to the host was often considerable after emergence of the parasite but never before emergence. Before emergence host growth was stimulated. This may have been due to stimulation by the parasite or the introduction of a stimulatory factor with the dead part of the inoculum (seed pods). The absence of pre-emergence loss and the considerable post emergence loss point to hydroparasitism as one of the major causes of loss to the host.

4. With a very heavy inoculum of parasite seeds pre-emergence loss to the host occured. This may have been due to the taking of elaborated material.

5. Parasites continued to grow under light-proof covers after emergence showing they continued to take eleborated material from the host after emergence; but the growth rate fell more rapidly and was more variable than parasites receiving light.

5. Under conditions of mineral deficiency (–N, –P, and –K) the hosts were able to support a greater relative weight of parasite without undergoing increased loss. If hydroparasitism is the chief cause of loss this would be expected since the slow-growing deficient plants would have lower water requirements, other factors being limiting. The absolute numbers of parasites were significantly less, however, with –N and –P.

7. Increasing the level of a balanced nutrient medium reduced loss to the host but did not reduce growth of the parasite.

8. A high level of $Ca(NO_3)_2$ in the medium reduced the number of parasites.

9. High levels of NaCl, $KH_2PO_4$ and $MgSO_4$ did not affect the loss caused to the host, but $KH_2PO_4$ increased the weight of parasite somewhat, as did $MgSO_4$ to a lesser extent.

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# SOME PHYSICAL PROPERTIES OF POT PLANT COMPOSTS AND THEIR EFFECT ON PLANT GROWTH

## II. AIR CAPACITY OF SUBSTRATES

by A. C. BUNT

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Previous investigation on the physical properties of pot plant composts and the use of bulky physical conditioners (Bunt [4]), showed that the inclusion of peat resulted in an increase in both plant growth and in the values of several of the physical properties of the composts, chiefly the macro-pore and air-capacity content. The use, however, of bulky conditioners, such as peat and grit, appreciably alters the chemical and biological properties of the substrate, and for this reason it has not been possible to attribute the improved growth solely to the change in physical properties.

Although the physical, chemical, and biological properties of soils are interrelated, one method of achieving a range of physical values with the minimum of chemical and biological changes is by the use of sand or soil separates. This technique enables a wide range of pore sizes and air capacities to be obtained within the basic substrate material.

Page and Willard [11] found that the air-space porosity and degree of aggregation gave the best physical assessment of soils for plant growth. Optimal air capacities and soil-separate sizes have been reported by various workers, (Agapov and Morgunov [1], Baver and Farnsworth [2], Erickson and Lemon [8], Page and Willard [11], and Yoder [13]). Russell [12] concluded that an air capacity of at least 10 per cent, and preferably 20 per cent, is required for field crops. Most of the investigators mentioned worked

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either in the field or with soil in large containers, and their conclusions would not necessarily apply to small containers, *e.g.* the 3½-inch-diameter pots commonly used for commercial tomato propagation in Britain. Hagin [9] reported an interaction between pot size and soil-aggregate size, and Cook and Millar [6] concluded that 1- to 2-gallon containers were too small to permit comparison of greenhouse and field-plot experiments.

The purpose of this study was first, to determine the effect of a range of air capacities, within a given substrate, on plants grown in small pots under the conditions applying to commercial glasshouses where no practical control of water tension is at present possible, and second, to ascertain to what extent, if any, the optimal air capacity of pot-plant substrates differs from that of field soils.

## MATERIALS AND METHODS

The sand and loam separates were obtained by putting the substrates through a series of sieves with mesh ranging from 0.25 mm. to 9.25 mm. A coarse silica sand, as normally used in making the John Innes composts, was chosen. It was leached several times before use, and was free from clay and other extraneous matter. The plants were grown in standard 3½-inch-diameter clay pots (approximately 3½ inches deep) arranged in randomised block design and irrigated as required with Hoagland's nutrient solution, sufficient solution being given at each application to cause leaching.

The soil used to provide the separates had a good aggregate stability, having been under a ley for at least 15 years and very probably for more than 60 years. It had the following mechanical analysis –

| | | |
|---|---|---|
| Clay | 21.2% | Total nitrogen 0.233% |
| Silt | 13.9% | |
| Fine Sand | 49.5% | pH 5.8 (adjusted to 6.5) |
| Coarse Sand | 7.7% | |

Air capacity and water retention of the sand and soil separates were determined in cans of 10 cm depth having a perforated zinc base covered with a disc of filter paper. Six cans were loosely filled with each separate and, to bring about natural compression, were irrigated daily for six weeks from a watering can fitted with a 'rose'. The cans were then slowly flooded and allowed to drain for 48 hours. Evaporation was prevented by fitting lids to the cans. Total pore space, air capacity and retained water were calculated from the true and apparent specific gravities. The specific gravities of the sand and the soil were determined by the British Standards Institution procedure; pF or desorption curves of the loam separates were obtained from undisturbed cores placed on sintered glass plates using a technique

similar to Croney *et. al.*[7] The undisturbed cores, 2 cm deep and 5.1 cm in diameter, were cut from separates in boxes which had been irrigated daily for six weeks as above. The pF curves and similar data have been expressed on a volume basis.

Because of limited drying facilities it was necessary, where large numbers of plants were grown to an advanced stage, to use fresh weight as the criterion of growth. Dry-matter determinations were made on a random sample of each treatment. Where plants were scored for the length of the period to flowering, anthesis was taken as the first flower to open on each plant.

Nitrogen, phosphorus, and potassium determinations on the oven-dry tissues were based on Cavell's [5] method, the digest being modified to include nitrates. Nitrogen-mineralisation rates were determined by the method of Bremner and Shaw [3].

## EXPERIMENTAL

### *Container depth*

The depth of substrate is known to influence the relative amounts of air and water retained after irrigation. The extent to which variation in the depth of containers normally used in horticulture affects the air capacity of two sand separates was determined. With increasing depth of separate, air capacity was found to in-

TABLE 1

The effect of container depth on air capacity and water retention

| Container depth | Fine separates < 0.5 mm | | Coarse separates 2.0–3.0 mm | |
|---|---|---|---|---|
| | Air capacity | Water | Air capacity | Water |
| 5 cm | 4.1 | 38.8 | 17.8 | 30.4 |
| 10 cm | 4.8 | 38.4 | 29.6 | 18.7 |
| 15 cm | 5.7 | 37.8 | 34.0 | 14.9 |
| 20 cm | 7.5 | 36.8 | 35.5 | 13.5 |
| 25 cm | 10.2 | 34.4 | 36.6 | 12.4 |

crease and water retention decrease in the manner shown in Table 1. The 5-cm depth corresponds to a 2-inch seed tray and the 25-cm depth to a 10-inch pot.

### *Sand separates*

*Antirrhinum orontium* and *Lycopersicon esculentum*, 'Potentate', two inbreeding species with different growth rates, were chosen to study the effect of different air capacities within the substrate.

TABLE 2

| Some physical properties of the sand separates | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Separate size, mm . . . | < 0.5 | > 3.0 | 0.5–1.0 | Control | 1.0–2.0 | 2.0–3.0 | 2.0–3.0 | 3.0–5.0 |
| Air capacity | 2.9 | 4.0 | 6.7 | 13.2 | 15.8 | 20.8 | 27.1 | 29.7 |
| Water retained | 38.5 | 34.0 | 34.9 | 25.5 | 28.9 | 25.4 | 19.0 | 16.2 |
| Total pore | 41.3 | 38.1 | 41.6 | 39.0 | 44.7 | 45.5 | 46.3 | 45.9 |
| Volume weight | 1.55 | 1.64 | 1.54 | 1.62 | 1.46 | 1.45 | 1.42 | 1.43 |

Some physical properties of the sand separates are given in Table 2.

*Antirrhinum orontium* was sown on April 22nd, and eight seedlings per treatment were pricked out on May 5th. The plants were scored for the number of days to anthesis and also for vegetative growth (weight) at two stages. At both stages there resulted an increase in growth with increasing air capacity up to approximately 25 per cent, and a small but statistically significant delay in anthesis (Table 3).

TABLE 3

| Growth and flowering of *Antirrhinum* in substrates of different air capacities | | | | | | | |
|---|---|---|---|---|---|---|---|
| Air capacity . . . | 2.9 | 4.0 | 6.6 | 15.8 | 27.3 | 29.7 | L.S.D. ($P = 0.5$) |
| 1st cutting Fresh wt. . . . . | 7.56 | 8.25 | 7.93 | 10.06 | 11.5 | 9.37 | 2.3 g |
| 2nd. cutting Fresh wt. . . . . | 29.0 | 31.18 | 32.68 | 44.44 | 41.81 | 35.50 | 4.9 g |
| Days to anthesis . | 26.5 | 26.75 | 26.5 | 27.0 | 28.0 | 28.62 | 0.63 days |

In the case of tomato, seeds of 'Potentate' were sown on July 23rd and forty seedlings per treatment were pricked out on July 31st. To determine the effect of the air capacity of the substrate at different stages of growth 10 plants from each treatment were cut and weighed at 14, 21, and 32 days after pricking out. The remaining 10 plants in each treatment were grown until they could be scored for the number of leaves produced before the first inflorescence, and for the number of days to anthesis. The results (Fig. 1) show that (i) an air capacity of approximately 25 per cent was optimal for vegetative growth, (ii) at air capacities above 25 per cent growth decreased, and (iii) this decrease was more apparent as the plants grew larger. High negative correlations were found

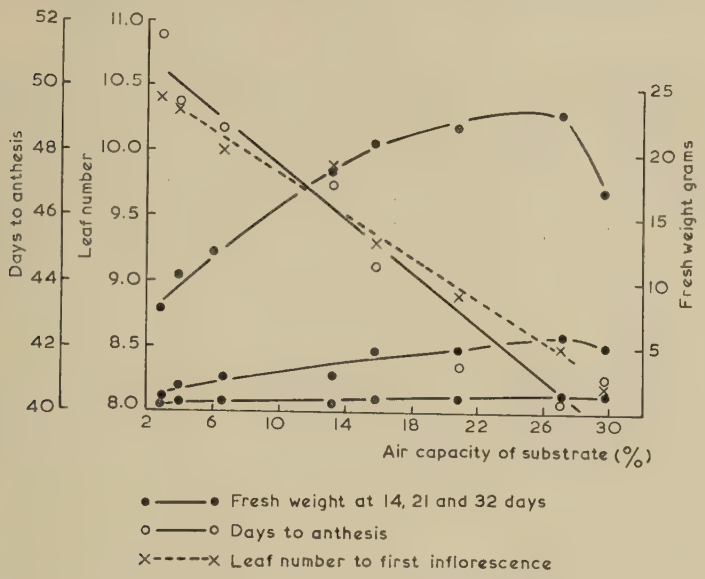
Days to anthesis
Leaf number
Fresh weight grams
Air capacity of substrate (%)
Fresh weight at 14, 21 and 32 days
Days to anthesis
Leaf number to first inflorescence

Fig. 1. The relation of the air capacity of sand separates on the growth and development of tomato 'Potentate'.

between (a) the number of leaves produced before the first inflorescence and the air capacity of the substrate, and (b) the number of days before anthesis and the air capacity of the substrates. The observed relationships were: –

(a) $y = -0.0806x + 10.65$ leaves $\quad r = -0.72$ ($P$ 0.001) (79 df.)

where $y$ = leaf number and $x$ = air capacity. The L.S.D. at the 5% level of the treatment means was 0.34 leaves

(b) $y = -0.4059x + 51.59$ days $\quad r = -0.85$ ($P$ 0.001) (79 d.f.)

where $y$ = days to anthesis and $x$ = air capacity. The L.S.D. at the 5% level of the treatment means was 2.04 days.

The plants from the third cutting, excluding the roots, were analysed for nitrogen, phosphorus and potassium content (Table 4). The phosphorus content of the plants, on a dry-matter basis, was constant for all treatments, whereas the nitrogen and potassium were negatively correlated with the air capacity of the substrates, the values being $r = -0.96$ ($P$ 0.001) for nitrogen and $r = -0.95$ ($P$ 0.0001) for potassium.

TABLE 4

| Mineral composition of tomato plants grown in sand separates of different air capacities (% of dry matter) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Air capacity of substrate . . . . | 2.9 | 4.0 | 6.6 | 13.2 | 15.8 | 20.8 | 27.1 | 29.7 |
| N | 4.00 | 3.91 | 3.91 | 3.81 | 3.66 | 3.49 | 3.19 | 3.01 |
| P | 0.275 | 0.270 | 0.213 | 0.275 | 0.287 | 0.263 | 0.260 | 0.245 |
| K | 3.9 | 3.7 | 3.3 | 3.3 | 3.0 | 3.0 | 2.7 | 2.6 |

*Soil separates*

A pilot experiment was made using a number of soil separates which gave a range of air capacities from 3.6 to 38.4 per cent (Table 5). Following the customary procedure when making up

TABLE 5

| Some physical properties of soil separates | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Separate size, mm . . | <0.25 | <0.5 | <1.0 | 0.5–1.0 | 1–2 | Control | 2–3 | 3–5 | 5–7.5 | 7.5–9.25 |
| Air capacity % . . . | 1.9 | 3.6 | 4.7 | 6.0 | 15.2 | 18.4 | 26.0 | 31.9 | 34.9 | 38.4 |
| Available water* % . | 45.4 | 47.9 | 45.5 | 46.2 | 41.1 | 34.5 | 31.3 | 24.9 | 20.6 | 16.5 |
| Macro porosity % . . | 8.8 | 21.0 | 26.0 | 40.3 | 39.3 | 30.9 | 41.4 | 38.2 | 43.2 | 48.3 |
| Total porosity % . . | 61.5 | 65.4 | 64.3 | 66.4 | 70.6 | 64.4 | 71.6 | 70.9 | 69.7 | 69.1 |
| Volume weight . . . | 0.97 | 0.87 | 0.90 | 0.84 | 0.74 | 0.83 | 0.72 | 0.73 | 0.76 | 0.78 |

* Available water = volume between air capacity and permanent wilting point.

pot composts, a base fertilizer having an analysis of N (organic) 5.1, P 3.2, and K 8.1 per cent was added to the separates at the rate of 6.5 g per litre (*ca* JIP[2] * level) prior to pricking-out the seedlings. Tomato, Potentate, was grown in 3½-inch pots in randomised-block design with eleven single-plant replicates. The seedlings were pricked out on September 10th and cut after 28 days. The results (Table 6) show that the best growth occurred with the smallest separates , < 0.5 mm having an air capacity of 3.6 per cent. A negative correlation of −0.65, (79 d.f.), between fresh weight and air capacity was obtained. This result was at variance with that obtained with the sand separates irrigated with nutrient solution. To determine the effect of the frequent application of liquid

* Footnote: JIP[2] = John Innes Potting Compost with two doses of base fertilizer.

TABLE 6

| Fresh weight of tomato 'Potentate' grown in soil separates or different air capacities | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Separate size, mm . . | <0.5 | 0.5–1.0 | 1.0–2.0 | Control | 2.0–3.0 | 3.0–5.0 | 5.0–7.5 | 7.5–9.25 |
| Air capacity . . . . % | 3.6 | 6.0 | 15.2 | 18.4 | 26.0 | 31.9 | 34.9 | 38.4 |
| Mean fresh weight* . g | 15.4 | 14.4 | 13.7 | 13.4 | 11.6 | 12.7 | 9.3 | 7.8 |
| Dry matter . . . . % | 7.0 | 7.5 | 7.4 | 7.7 | 7.5 | 7.3 | 7.9 | 8.2 |

* L.S.D. $P = 0.5\%$; = 2.03 g.

fertilizer as opposed to a single application to the substrate before filling the pots, a further experiment was made in which half the plants had base fertilizer added as before and were then irrigated with water, whilst the remainder had only phosphorus added prior to pricking out and, at each irrigation, received a nutrient solution having the composition, $NO_3$—403, $NH_4$—27, P—31, K—117, Mg—24 ppm. Eight plants from each treatment were cut and weighed 28 days after pricking out and the remainder were grown on and scored for the period to anthesis, number of leaves produced before the first inflorescence, number of flowers in the first inflorescence, and mineral composition.

In the previous experiment, growth progressively increased as the air capacity of the substrate was reduced to 3.6 per cent. It was decided, therefore, to examine the effect on plant growth of substrates with lower air capacities by extending the range of air capacity down to 1.9 per cent by the use of <0.25 mm separates. Tomato, 'Potentate', was pricked out on April 2nd, and the experiment was laid down in two adjacent randomised blocks. It was considered inadvisable to grow both nutrient treatments in one large randomised block because of the risk of splashing nutrient solution on to the base fertiliser treatments. The fresh weights of the plants harvested 28 days after pricking out are shown in Fig. 2. A clear contrast in growth patterns is apparent between the nutrient and base fertiliser treatments with respect to the extremes of air capacity. With the remaining half of the plants the air capacity of the substrate had no significant effect on either the number of leaves produced before the first inflorescence, the number of flowers or the nitrogen, phosphorus, and potassium contents of the plants. The effect of air capacity on the period to anthesis is shown in Fig. 3.

The depression in growth at the lowest air capacity in the base

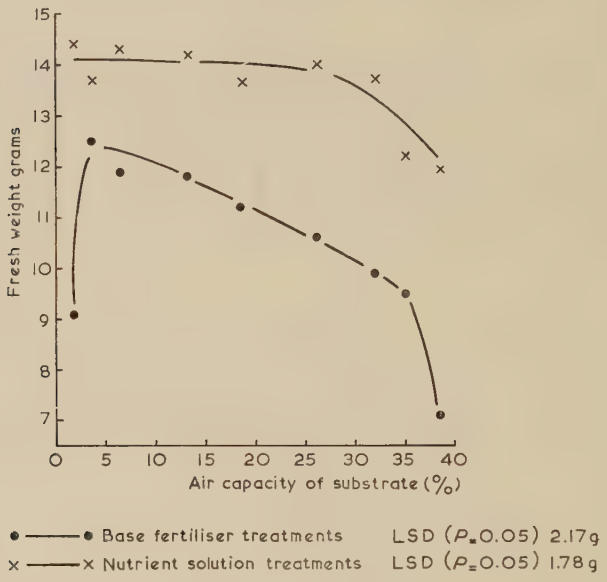


Fig. 2. Fresh weights of tomato 'Potentate' grown in loam separates of differing air capacities.

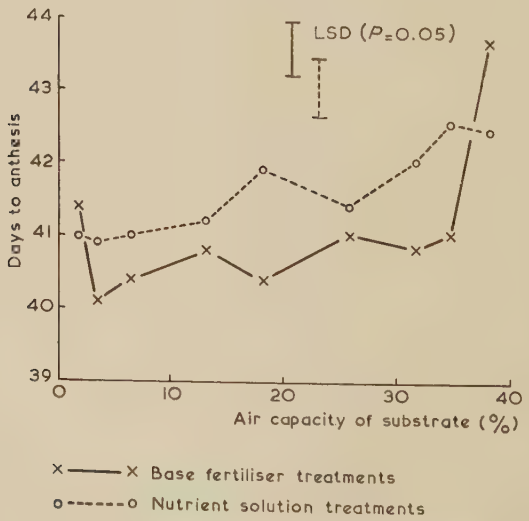


Fig. 3. The effect of the air capacity of soil separates on the period to anthesis of tomato 'Potentate'.

fertilizer treatments (Fig. 2) could have been caused by the lack of mineralisation of the organic nitrogen (hoof and horn) in the base fertilizer. To test this possibility the mineralisation rate of hoof and horn in two treatments, *viz* separates < 0.25 mm with an air capacity of 1.9 per cent and the control soil, air capacity 18.4 per cent, was determined in samples which were incubated in the laboratory at field capacity. The nitrogen values in the two treatments were: separates 0.067 mg and control loam 0.208 mg.

The desorption curves of some of the smaller separates are given in Fig. 4. Each curve is the mean of four replicates.

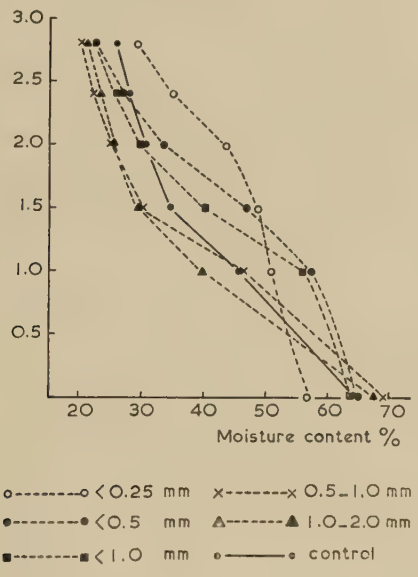


Fig. 4. Desorption curves of some soil separates.

## DISCUSSION

The data presented on the vegetative and developmental response of the tomato to a range of air capacities show a marked contrast between the sand and soil substrates. Under the system of cultivation adopted in these experiments increasing air capacity of the sand substrate up to approximately 25 per cent resulted in a higher growth rate. Beyond this value growth declined. This was probably due to the low quantity of water retained by the substrate,

the plants in the coarse separates were the first to show a water deficit, and under very high solar radiation they required irrigation as frequently as every two hours.

With the loam separates, growth in the base fertilizer treatments decreased with increasing air capacity above 5 per cent. Whilst no study was made of the leachate from the pots, it would appear from comparison of the growth curves of the nutrient and base fertilizer treatments that the depression in growth over the range of 5 per cent to 25 per cent air capacity was primarily due to the loss of nutrient by leaching. Probably poor contact between the roots and the soil solution, and higher water tensions were additional factors at the higher air capacities. Roots were observed to be growing around the periphery of the large separates rather than permeating into the separates. From further comparison of the growth curves, over the ranges from 0 to 5 per cent ,and from the data obtained on nitrogen mineralisation in the finest separates ($< 0.25$ mm), it seems that the depression in growth at air capacities below 5 per cent in the base fertilizer treatment series was primarily due to lack of available nitrogen rather than the direct effect of too low an air capacity. This suggests that with composts having low air capacities the use of some mineral nitrogen rather than only organic supplementary nitrogen might be advisable.

The air capacity of the soil separates had much less effect on the development of the tomato than the air capacity of the sand separates. There was no significant difference in the number of leaves produced before the first inflorescence, and whilst the air capacity of the substrate had a statistically significant effect on the number of days to anthesis the maximum difference between treatments in the number of days to anthesis was less than four.

Whilst the data in these experiments have been interpreted in terms of air capacity it must be remembered that air capacity and available water are inversely related, and the extent to which different water tensions in the experiments have directly influenced plant growth cannot be assessed. Miller and Mazurak [10], working with sunflowers, have reported an interaction between the geometric mean diameter of separates and the water tension. There is at present, however, no suitable method of controlling the water tension of pot plants grown under commercial conditions where water is applied from a visual assessment.

It would appear from the results of these experiments that the increase in growth observed with tomato and *Antirrhinum* when peat is added to soils (Bunt [4]) is not due to the increase in either air capacity or macro-pore content, brought about by the peat, but rather to some inherent property of the peat. The optimal air capacity of 5 per cent for substrates receiving the customary base fertilizer is much below the reported optimal air capacity of 20 per cent for field soils.

From these experiments and other unpublished data on the nutrition of pot plants it is concluded that the application of a nutrient solution to a compost at each irrigation rather than a single application of base fertilizer when making the compost, is a method that merits further examination.

## SUMMARY

The growth and development of tomato, 'Potentate', in sand and soil separates having a range of air capacities has been studied.

With the sand separates, an air capacity of 25 per cent gave optimum growth. Both the number of days to anthesis and the number of leaves produced before the first inflorescence were negatively correlated with air capacity.

Tomatoes grown in soil separates with base fertilizer added before pricking-out showed an optimum air capacity requirement of 5 per cent. Plants in similar substrates and receiving a nutrient solution at each irrigation were less responsive to differences in air capacity over the range 1.9 to 20 per cent. Differences in the air capacity of the substrate had no effect on the number of leaves produced before the first inflorescence and little effect on the number of days to anthesis.

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# EFFECT OF ORGANIC MATTER ON THE DEVELOPMENT IN SOIL OF ANTIBIOTIC-PRODUCING MICRO-ORGANISMS

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## INTRODUCTION

The capability of many soil micro-organisms to produce antibiotics has suggested that this may be in some cases a factor in the maintenance or change of the microbiological balance in soil, and thus indirectly concerned with soil fertility. The majority of antibiotic-producing micro-organisms are normal soil inhabitants. Great quantities of antibiotics can be extracted from their cultures on laboratory media, yet antibiotics have not been detected or have been found only in small amounts in soil. To increase their production in soil various methods have been adopted. The most common are the inoculation of soil with antagonists [13 15 22] supplementing the soil with organic matter [4 9 11 12 21] and a combination of the two [2 16 23].

Antibiotic production in soil appears to be favoured by the presence of organic matter. It was shown [7] that the occurrence of antagonistic streptomyces in soils is apparently correlated to the state rather than to the type of soil, and that the percentage of antagonists seems to increase with the increase of organic matter. The purpose of the present study was to investigate to what extent organic matter additions to soil affect the development of antagonistic and non-antagonistic streptomyces and other bacteria. The supplements were limited to two groups, simple nutrients, glucose, sucrose and peptone, and complex organic materials, wheat straw, clover, cotton-seed cake and sheep manure (composted mixture of straw and sheep urine and faeces).

## MATERIALS AND METHODS

The soil used was a light clay loam of pH 7.7 with C/N 5.6, containing 0.79% carbon and 0.14% nitrogen, taken from a field used for growing vegetables in the vicinity of the Faculty of Agriculture, Giza.

The soil was air dried, passed through a 3-mm sieve and portions were added, as percentages (w/w) of the air-dry soil:

a) 1% glucose
b) 0.5% sucrose
c) 0.5% peptone
d) 2% wheat straw and 0.5% sodium nitrate
e) 5% clover
f) 1% cotton seed cake
g) 2% sheep manure
h) 0.5% sodium nitrate
i) No addition (control)

The moisture was adjusted to 50% water-holding capacity with sterile distilled water. The calculated amounts of the supplements were either dissolved in the water or added directly to the soil. Each portion of soil was then thoroughly mixed and 100-g quantities were distributed in 250-ml erlenmeyer flasks which were weighed, and incubated at 30°C. The flasks were weighed regularly and the original weight was restored by adding water.

Plate counts of antagonists and non-antagonists were made after 0, 3, 10, 20, and 30 days in the case of the simple nutrients and after 0, 10, 20, 30 and 40 days in the case of complex organic substances. Preliminary trials showed that the highest counts of antagonists were obtained from the low dilutions $25 \times 10^{-3}$ and $25 \times 10^{-4}$. Antagonists were distinguished by the clear zones surrounding colonies. They were marked after 7 days' incubation at 30°C and the plates were kept under daily observation until the final counts were recorded. Total counts of streptomyces and of other bacteria (hereafter referred to as "*bacteria*") were made after 15 days at 30°. In all cases the counts represent the average of four plates.

Thornton's asparagin—mannitol agar [18], Talyor's tryptone—yeast—soil extract agar [17] and Topping's yeastrel-peptone agar [19] in which the yeastrel was replaced by yeast-act (a commercial yeast autolysate) were compared as plating media in preliminary trials. The results were in favour of the modified Topping medium, on which the highest counts were recorded and the colonies of the antagonists were most clearly defined. This medium was therefore used throughout.

## RESULTS

### *Soil with no addition*

Fig. 1 shows the results obtained from five specimens of soil, which were used as controls in examining the effect of the additions. Difference between counts of total bacteria, total streptomyces, antagonistic bacteria, and antagonistic streptomyces proved to be statistically non-significant (0.05 level) within the samples and the

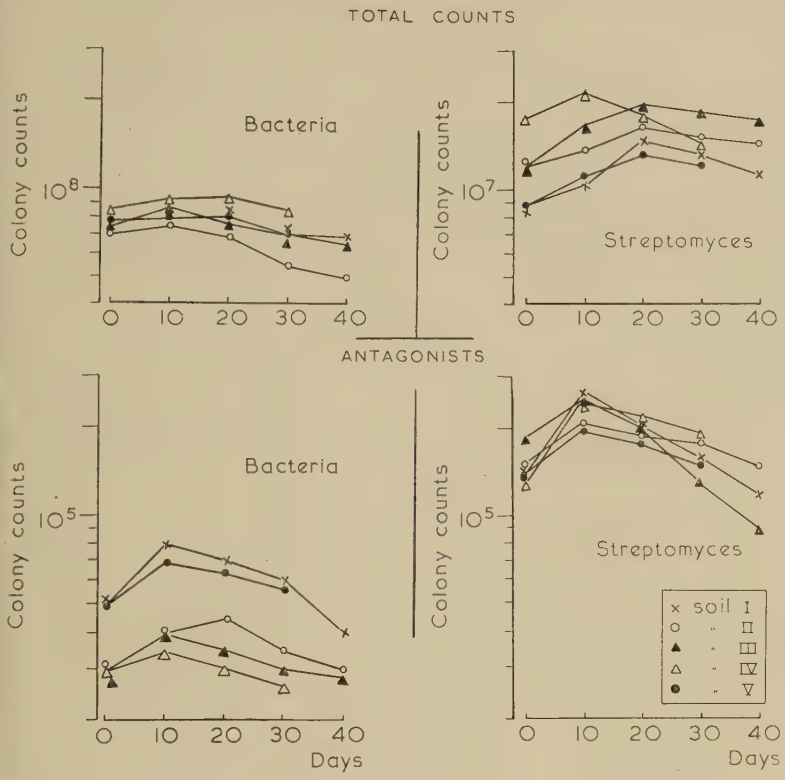


Fig. 1. Changes in total and antagonistic colony counts in unsupplemented soils.

various periods of incubation. Although the counts of streptomyces were much lower than those of bacteria, they markedly exceeded the bacteria in the numbers of antagonistic types.

*Effects of the simpler nutrients*

The effect of sugars and peptone on total and antagonist counts is shown in Fig. 2. The total counts of streptomyces were only slightly affected by the addition of glucose or sucrose but peptone induced a marked increase. The counts of antagonistic streptomyces, on the other hand, considerably increased on the addition of the sugars as well as of peptone, the latter, however, being the most effective. It is of interest that all the rises in count promoted by the nutrient additions occurred mainly in the first 10 days.

The multiplication of the antagonists is compared with that of

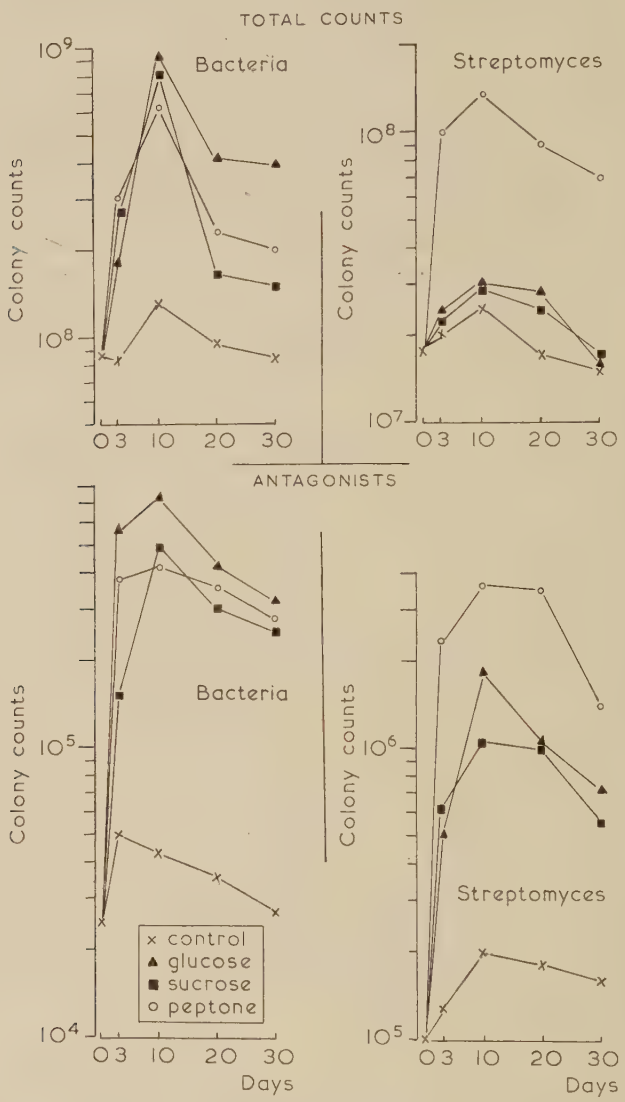


Fig. 2. Effect of the simpler nutrients on total and antagonistic colony counts.

the other organisms in Table 1. The three amendments obviously induced a greater increase in the antagonists – bacteria as well as streptomyces – than in the non-antagonists. Bacteria and streptomyces, however, did not respond in the same way to the sugars

TABLE 1

Effect of simple nutrient additions to soil on the multiplication of antagonists and non-antagonists

| Treatment | Organism | Initial count $10^{-6}$g | Multiplication factor * after | | | |
|---|---|---|---|---|---|---|
| | | | 3 | 10 | 20 | 30 days |
| *Non-antagonists* | | | | | | |
| Control | Bacteria | 84.98 | 1.0 | 1.6 | 1.1 | 1.0 |
| | Streptomyces | 17.40 | 1.1 | 1.5 | 0.9 | 0.9 |
| Glucose | Bacteria | | 2.2 | 11.2 | 4.9 | 4.7 |
| | Streptomyces | | 1.4 | 1.6 | 1.6 | 0.8 |
| Sucrose | Bacteria | | 3.2 | 9.5 | 3.1 | 3.0 |
| | Streptomyces | | 1.2 | 1.6 | 1.4 | 1.0 |
| Peptone | Bacteria | | 3.6 | 7.4 | 2.7 | 2.4 |
| | Streptomyces | | 5.6 | 7.7 | 5.0 | 3.9 |
| *Antagonists* | | | | | | |
| Control | Bacteria | 0.03 | 1.7 | 1.4 | 1.0 | 2.0 |
| | Streptomyces | 0.10 | 1.1 | 2.4 | 2.2 | 1.8 |
| Glucose | Bacteria | | 23.0 | 30.0 | 17.0 | 13.0 |
| | Streptomyces | | 5.0 | 18.3 | 10.0 | 7.3 |
| Sucrose | Bacteria | | 6.0 | 20.0 | 12.0 | 10.0 |
| | Streptomyces | | 9.3 | 10.0 | 11.0 | 5.0 |
| Peptone | Bacteria | | 15.0 | 16.5 | 14.4 | 11.5 |
| | Streptomyces | | 23.3 | 35.5 | 36.2 | 14.0 |

* Multiplication factor $= \sim \frac{\text{Count at time of sampling}}{\text{Initial count}}$

and the peptone. While the sugars were particularly favourable for the development of bacteria, the peptone stimulated a greater increase in the streptomyces.

The quantitative changes in the antagonists and non-antagonists may or may not result in disturbances of any possibly existing equilibrium, *i.e.* ratio of antagonists/total counts, despite the apparent marked increases recorded for both. Fig. 3 shows the changes in antagonists/total counts in the bacteria, streptomyces and total bacterial population. A rise in the curves indicates a shift in the equilibrium in favour of antagonists. The results have been submitted to statistical analysis to determine the significance of the changes at the 5% level. It is quite apparent that the observed disturbances due to the additions were in favour of the antagonists, the most significant disturbance occurring among the streptomyces and the least among the bacteria. The individual additions did not

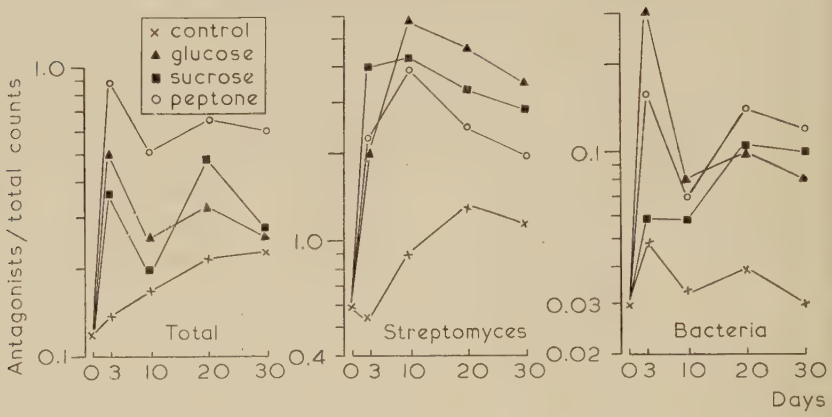


Fig. 3. Effect of the simpler nutrients on the ratio of antagonist to total counts.

result in the same degree of disturbance among the streptomyces and other bacteria. While all additions incited significant increases in the ratio of antagonists among the streptomyces, glucose as well as sucrose increased the ratio more significantly than peptone This may be explained by the fact that sugars caused hardly any increases among the non-antagonistic streptomyces while peptone favoured both types. When bacteria alone are considered the only significant disturbance was recorded in the case of glucose, especially after 3 days. The ratio of total antagonists to total counts was only significantly disturbed in the case of peptone. In spite of significant increases in the ratios of antagonists to total streptomyces counts stimulated by the sugar additions, no significant disturbance in the ratio of total antagonists to total counts could be detected. This is obviously due to the fact that the counts of the bacteria were considerably higher than those of streptomyces, and therefore the significant increases in the antagonistic streptomyces were masked.

*Effect of complex organic matter*

All the organic supplements induced marked increase in the counts generally, antagonists, and total counts, whether bacteria or streptomyces, throughout the observation period (Fig. 4). Sodium nitrate alone did not result in any noticeable changes. When the rate of increase of both antagonists and non-antagonists, *i.e.*

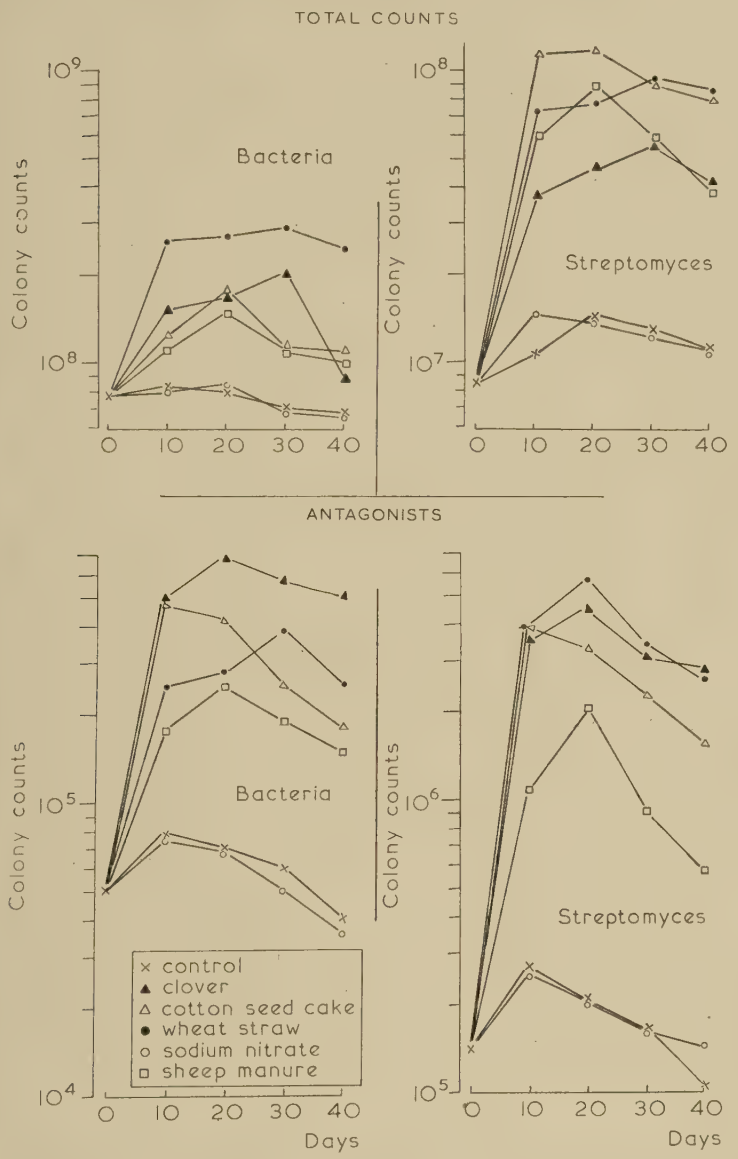


Fig. 4. Effect of complex organic matter on total and antagonist colony counts.

the multiplication factor, is considered (Table 2), it will be noticed that the organic supplements obviously induced a more marked rate of increase in the antagonists – bacteria as well as strepto-

TABLE 2

Effect of adding complex organic substances to soils on the multiplication of antagonists and non-antagonists

| Treatment | Organism | Initial count $10^{-6}$g | Multiplication * factor after | | | |
|---|---|---|---|---|---|---|
| | | | 10 | 20 | 30 | 40 days |
| *Non-antagonists* | | | | | | |
| Control | Bacteria | 77.45 | 1.1 | 1.0 | 0.9 | 0.9 |
| | Streptomyces | 8.66 | 1.3 | 1.7 | 1.5 | 1.3 |
| 0.5% $NaNO_3$ | Bacteria | | 1.2 | 1.2 | 1.0 | 0.9 |
| | Streptomyces | | 1.7 | 1.2 | 1.0 | 0.9 |
| 2% wheat straw + 0.5% $NaNO_3$ | Bacteria | | 3.4 | 3.5 | 3.5 | 3.3 |
| | Streptomyces | | 8.2 | 8.6 | 11.0 | 10.9 |
| 5% clover | Bacteria | | 2.2 | 2.5 | 3.0 | 1.4 |
| | Streptomyces | | 2.8 | 3.6 | 4.4 | 3.4 |
| 1% cotton seed cake | Bacteria | | 1.7 | 2.4 | 1.6 | 1.6 |
| | Streptomyces | | 9.7 | 10.3 | 7.6 | 6.7 |
| 2% sheep manure | Bacteria | | 1.5 | 2.1 | 1.5 | 1.4 |
| | Streptomyces | | 5.0 | 7.9 | 4.9 | 3.3 |
| *Antagonists* | | | | | | |
| Control | Bacteria | 0.05 | 1.6 | 1.4 | 1.2 | 0.8 |
| | Streptomyces | 0.14 | 1.9 | 1.4 | 1.1 | 0.7 |
| 0.5% $NaNO_3$ | Bacteria | | 2.0 | 1.7 | 1.0 | 0.7 |
| | Streptomyces | | 1.7 | 1.3 | 1.1 | 0.9 |
| 2% wheat straw + 0.5% $NaNO_3$ | Bacteria | | 5.0 | 5.6 | 7.8 | 5.0 |
| | Streptomyces | | 28.6 | 41.4 | 24.1 | 17.9 |
| 5% clover | Bacteria | | 16.7 | 22.7 | 19.0 | 16.7 |
| | Streptomyces | | 23.3 | 29.5 | 20.0 | 18.3 |
| 1% cotton seed cake | Bacteria | | 16.7 | 14.3 | 8.3 | 6.0 |
| | Streptomyces | | 44.4 | 36.1 | 25.1 | 16.7 |
| 2% sheep manure | Bacteria | | 6.0 | 8.3 | 6.3 | 5.0 |
| | Streptomyces | | 12.0 | 15.3 | 10.0 | 4.0 |

* $\frac{\text{Counts at time of sampling}}{\text{Initial count}}$

myces – than in the non-antagonists. The antagonistic streptomyces showed a more pronounced rate of development than the antagonistic bacteria.

The effect of soil additions on the ratio between antagonists and total counts is shown in Fig. 5. The first point to be noticed is that both the control soils and soil supplemented with sodium nitrate

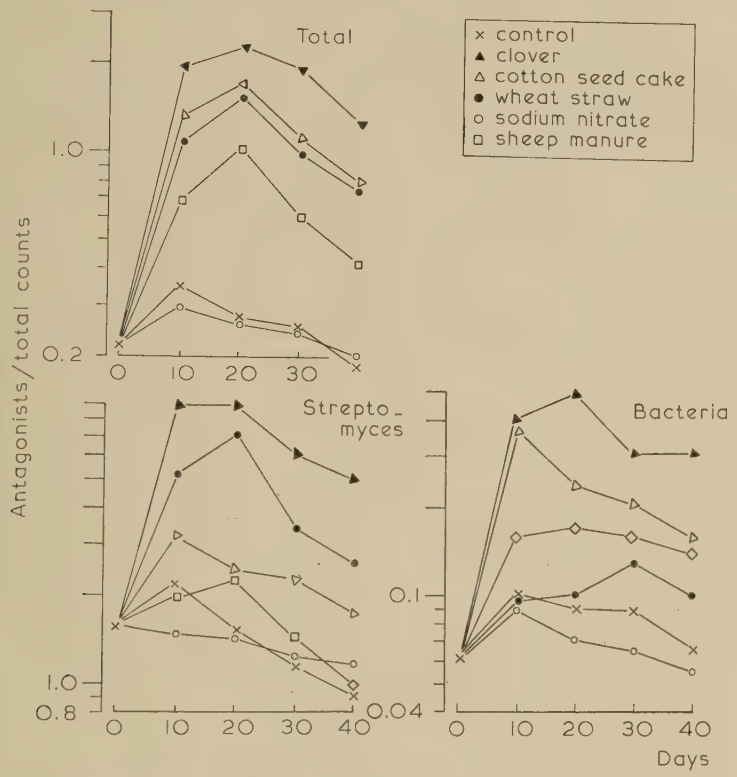


Fig. 5. Effect of complex organic matter on the ratio of antagonist to total counts.

appeared to be in a fairly stable state of equilibrium – bacteria, streptomyces, and total counts – as no significant changes took place during the experimental periods. In the case of clover, cotton-seed cake and wheat straw the increases in total antagonists resulted in significant disturbance in the ratio during the whole observation period, *i.e.* up to 40 days, the degree of disturbance increasing significantly during the first 20 days after which period it decreased. Clover caused a significantly more marked effect than the other supplements during the first 20 days. Regarding the streptomyces, clover, and wheat straw were the only materials that caused significant disturbance in the ratios during the first 20 days. In contrast the disturbance within the bacteria was significant in the case of clover and cotton seed cake.

## DISCUSSION

The development of micro-organisms in soil is mainly dependent on the presence of available carbonaceous materials, which serve as sources of energy. Mineral nitrogen supplements like sodium nitrate do not materially affect the growth in soils poor in organic matter and having a narrow C/N ratio as is commonly the case in Egyptian soils. Under conditions favouring rapid multiplication, antagonistic types of organisms might be expected to have a greater chance of increasing than the non-antagonists. In trials to combat soil-borne plant pathogens by means of antibiotic-producing micro-organisms, the best results were usually obtained when organic matter was added, *i.e.* under conditions inducing rapid multiplication. The effect could be due either to increased antibiotic production or to a greater increase of antagonistic organisms.

Antagonism in soil through the accumulation of antibiotics is rather doubtful in the face of existing evidence [1 3 13]. It is difficult to detect antibiotics in soil. Pure antibiotics added to soil may be quickly inactivated through adsorption on soil particles [8 13 14] and decomposition by micro-organisms [1 3 5 10].

It seems rather more likely that antagonism is enhanced by favouring the multiplication of antagonists without regard to whether they are potent antibiotic producers or not. Most available data indicate that weak antibiotic producers are the more commonly met with in soils [6]. In fact it is easy to visualise the relative unimportance of strong or weak antibiotic capabilities of the organisms in the competitive growth in soil, if one assumes that most antagonistic action in soil takes place in the close proximity of the antagonists so that factors affecting the diffusion and accumulation of antibiotics hardly come into play. The results reported here appear to give some support to this idea. Whenever rapid multiplication of organisms was induced by the inclusion of energy sources in the soil, antagonistic types increased more than non-antagonists and the apparent equilibrium between the two types in the untreated soils was significantly disturbed in favour of antagonists.

Significant increases in the percentage of antagonists to total counts coincided with the period of maximum growth, a point worthy of note when antagonism is made use of in combating

soil-borne plant pathogens. Organic substances differed in their effect on the proportion of antagonists. Well-composted organic material (sheep manure) was the least effective.

Streptomycetes, though much less numerous than bacteria, contained a much higher percentage of antagonistic types. Organic substances appear to have a differential effect, some favouring the antagonistic streptomyces, others the antagonistic bacteria, the former being more common.

A point worth discussing concerns the limitations of the method employed in this study for counting anatagonists, the so-called crowded plate method [20]. This method undoubtedly lacks accuracy for at least two obvious reasons. The counts are taken from the low-dilution plates where the number of organisms is high and growth suffers from competition of space and nutrients. The formation of the clear zones indicating the antagonistic property depends on the type of the neighbouring organisms which in this case would be mainly aerobic spore-formers prevailing in the low dilutions. However, the method is still the only general straightforward simple method for counting antagonists in soil and in this study at least did yield quite regular results.

## SUMMARY

In soil incubated at 30°C no significant change in total counts of streptomyces, other bacteria, or antagonistic types of either group occurred during a 30 to 40-day observation period. Addition of 0.5% (w/w) sodium nitrate had no significant effect on the counts. Organic substances induced a rapid multiplication of micro-organisms, antagonistic types increasing more than others. The main increase in the percentage of antagonists coincided with the period of maximum growth. Organic substances differed in their effects. Some favoured the antagonistic streptomyces and some other antagonistic bacteria. Streptomyces contained the majority of the organisms that were shown to produce antibiotics.

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# FURTHER EVIDENCE FOR THE IMPORTANCE OF RESIDUAL LEAF PROTEIN COMPLEXES IN LITTER DECOMPOSITION AND THE SUPPLY OF NITROGEN FOR PLANT GROWTH

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## INTRODUCTION

It has previously been demonstrated (Handley [14]) that the fresh leaves of a number of plant species contain substances, soluble in water, which will precipitate proteins and have characteristics similar to those of vegetable tannins. It was suggested that when the leaves of these species die at the time of leaf fall the residual proteins of the mesophyll cells form complexes with the protein-precipitating substances. These complexes cover and probably permeate the cellulose walls of the mesophyll cells and, especially in the case of those species whose litter can give rise to raw humus, seem, from microscopical observations, to be resistant to decomposition thereby hindering the decomposition of both residual leaf proteins and the cellulose walls.

Although protein-precipitating substances are to be found in the leaves of species invariably associated with mull formation as well as in the leaves of species associated with mor formation the complexes formed by interaction between extracts of leaves of various species and gelatin were found to have differential rates of solubilization by micro-organisms especially when they have been air dried. Thus *Calluna vulgaris* leaf extract—gelatin complex was less readily solubilized than the *Chamaenerion angustifolium* leaf extract—gelatin complex.

If this resistance to solubilization is the basis of a resistance

to decomposition of residual leaf proteins in litter, it is likely to have important effects on the supply of mineral nutrients, especially N and P, for the growth of plants on sites where these resistant complexes occur and on other soil processes; a lack of readily assimilable organic nitrogen may also influence the species composition and biomass of the soil fauna.

An attempt has therefore been made to ascertain the relative nitrogen-supplying capacity of various model protein complexes using tree seedlings for the bioassay of the liberated nitrogen.

## MATERIALS AND METHODS

### (a) *Choice of tree species used for bioassay*

As Mikola [27] had found that the development of *Betula pubescens* seedlings in pure-culture experiments was very strongly influenced by the nitrogen concentration of the nutrient solution, and apparently more strongly influenced than was the growth of pine seedlings in similar experiments carried out by Björkman, it was decided to use *Betula pubescens* seedlings to assess the nitrogen-supplying capacity of the model protein complexes. Mikola's findings were subsequently supported by the experiments of Ingestad [18 19] who found that in water cultures a relatively high nitrogen requirement was the most outstanding characteristic of birch seedling nutrition. Birch seems to require larger supplies of mineral nutrients, and especially of nitrogen, than spruce to produce the same amount of dry matter.

### (b) *Choice of medium on which to grow birch seedlings*

The birch plants were grown on well-washed, lime free quartz sand. Soil was not used because of the complications which would probably have arisen due to mineralization of the nitrogen of the organic matter already present in soil although the rate of decomposition of the protein complexes may not have been the same in sand as it would have been in soil.

### (c) *Preparation of protein complexes*

Leaf extracts were prepared by placing freshly collected green leaves with twice their weight of distilled water, adding chloroform at the rate of 5% of volume of the distilled water, and keeping the mixtures in sealed jars, filled as completely as possible, for two weeks. At the end of this time the fluid was squeezed out by means of a press and the extract clarified by centrifuging. The extract was then kept in the presence of chloroform, in screw cap bottles completely filled with extract, until required for use.

The plant species whose leaves were used for the preparation of extracts were *Calluna vulgaris*, *Chamaenerion angustifolium* and *Circaea lutetiana*. These species were chosen as their leaf extracts give abundant precipitates

with gelatin and also because Calluna is generally associated with mor sites while Circaea is associated with mull sites; Chamaenerion occurs on mull sites and also on cleared and burned mor sites, where it may persist for some time, and has been considered to indicate the presence of appreciable supplies of nitrogen in the soil (Tamm [37]). It therefore seemed reasonable to suppose that protein complexes formed by extracts of the leaves of these species might have differential rates of breakdown and release of mineral nitrogen for plant growth, although it must be borne in mind that such deductions, based on field observations, may not be justified in the case of *Circaea lutetiana* if only because of the base-rich soil conditions which this species appears to prefer.

Although gelatin has been used for the detection of protein-precipitating substances in leaf extracts it was considered that casein would give complexes more readily manageable on a quantitative basis and therefore casein * was used for the preparation of the complexes.

Since the birch seedlings were to be grown in quartz sand contained in glass flowerpots it was considered that a suitable amount of casein to use for the formation of protein complex for each pot would be that which was equivalent, in order of nitrogen content, to the nitrogen content of the litter which might be expected to fall on an area equivalent to the surface area of the sand in a pot in one year. From calculations this appeared to be of the order of 0.05 g of nitrogen per pot which is contained in approximately 0.3 g of protein.

The protein complex to be added to each pot was prepared separately by gently warming 0.3 g of casein with 10 ml of distilled water and adding the mixture slowly with constant shaking to 100 ml of the previously prepared leaf extract. The mixtures were then allowed to stand for four weeks, in the presence of a little chloroform in flasks capped with a layer of polythene, to allow time for any oxidation, polymerization, and related processes to take place; the flasks were gently shaken at intervals of a few days. The complexes were separated by centrifuging and then washed several times with distilled water being separated by centrifuging after each washing. The complexes were then allowed to dry at room temperature and when dry were pulverized before being added to the sand. At this stage the complexes were dark brown in colour. When freshly precipitated the protein complex is very pale brown in colour and it is not known whether the change to a dark-brown colour is due to changes in the protein-precipitating factor itself or to changes in associated materials from the leaf which have become attached to the protein or protein-precipitating factor at some stage in the preparation of the complex.

Separate batches of the complexes were prepared for determination of their nitrogen contents by the Kjeldahl method in order to ascertain the amount of nitrogen added to each pot. Since control pots, to which untreated

* Casein supplied by British Drug Houses Ltd. and known as Light White Soluble was used.

casein was added, were set up the nitrogen content of the casein was also determined.

For comparative purposes pots were also set up in which the source of nitrogen was H-layer material from the $A_0$-horizon of a Calluna podzol. One gram of this material in an air dry condition was added to each pot. The nitrogen content of this material was also determined by the Kjeldahl method.

### (d) *Raising of birch seedlings*

The *Betula pubescens* seeds were placed on the surface of moist quartz sand in seed pans on May 1st. After germination they were allowed to grow in the seed pans until July 3rd when, although still quite small, they were large enough to be transplanted into the experimental pots. During growth of the seedlings in the seed pans 10 ml of the following solution of mineral nutrients was added at approximately weekly intervals to each seed pan. The solution is that used by Melin [26] except that glucose is omitted.

| | |
|---|---|
| $K_2HPO_4$ | 0.5 g |
| $CaCl_2$ | 0.05 g |
| NaCl | 0.025 g |
| $MgSO_4.7H_2O$ | 0.15 g |
| $(NH_4)_2HPO_4$ | 0.25 g |
| Fe-citrate (1% soln) | 1.2 ml |
| distilled water to 1 litre | |

Water was supplied to the seedlings by means of asbestos wicks going through the drainage holes of the seed pans, one end of the wicks being immersed in distilled water whilst the other was buried in the sand.

### (e) *Setting up the experimental pots*

Flowerpots of brown glass and 4″ internal diameter were used to contain the washed quartz sand. A multi-strand asbestos cord was knotted and the knot placed so that it rested over the drainage hole of the pot to prevent the sand running out. The separated strands of the asbestos cords were distributed evenly through the volume of the pot and 500 g of sand placed around them. The protein complex to be added to the pot was then mixed with 250 g of sand and the mixture placed around the strands of the asbestos cord and on top of the 500 g of sand already in the pot. Four birch seedlings were planted in each pot which was watered with distilled water; care being taken that water did not run out through the drainage hole. The pots were suspended by their rims through holes in the top of a box so that the lower parts were in darkness; the lower end of the asbestos cord protruding through the drainage hole was immersed in a beaker of distilled water so that water was supplied, by capillarity, to the birch seedlings as required.

The pots in the boxes were then placed in a single row running very nearly in an East-West direction in a Möller [29] house whose ridge was also orientated in an East-West direction. The light intensity to which the plants were exposed was approximately 50 per cent of full daylight.

There were four replicates (pots) of each treatment which were arranged in the following order: –

East West

$A_1$ $B_1$ $C_1$ $D_1$ $E_1$ $A_2$ $B_2$ $C_2$ $D_2$ $E_2$ $E_3$ $D_3$ $C_3$ $B_3$ $A_3$ $E_4$ $D_4$ $C_4$ $B_4$ $A_4$

Treatment A = Calluna raw humus (H-layer material).
B = Calluna caseinate
C = Circaea caseinate
D = Chamaenerion caseinate
E = Casein alone

In order that the seedlings should have adequate supplies of mineral nutrients other than nitrogen each pot was watered once a week with 10 ml of the following solution: –

| | |
|---|---|
| $K_2HPO_4$ | 0.375 g |
| $KH_2PO_4$ | 0.25 g |
| $CaCl_2$ | 0.05 g |
| NaCl | 0.025 g |
| $MgSO_4.7H_2O$ | 0.15 g |
| Fe-citrate (1% soln) | 1.2 ml |
| distilled water to | 1 litre |

This solution had a reaction of pH 6.95 and the asbestos wicks rendered the reaction less acid so that the root environment of the birch seedlings had a reaction slightly above pH 7.

### (f) *Harvesting of plants for analysis*

During the autumn the leaves were collected daily as they turned brown and fell off; the leaves from the plants of each pot being placed in a separate container. The leaves from each seedling of a pot could not be kept separate as often it was not known which seedling they came from.

After all the leaves had been harvested the seedlings were carefully removed from the pots and all sand washed away from the roots by a gentle stream of water. The length of the stem between the base of the terminal bud and the cotyledon scars was recorded before the stem was severed from the root at the cotyledon scars. These two parts of the seedlings were then dried at 50°C for 24 hours before being stored separately until the analyses could be carried out.

### (g) *Analysis of the seedling material*

The leaves from the seedlings in each pot and the roots and stem of each plant were dried at 105°C for dry-weight determination. In the case of the larger specimens the dried material was milled before samples were taken for determination of the nitrogen content by the Kjeldahl method. The total nitrogen content of the leaves from each pot of seedlings was determined

and also the nitrogen content of the stem and roots of each seedling separately. In the case of the smallest seedlings the whole of the stem and roots of each seedling were used, without being milled, for determination of the nitrogen content of the two parts of the seedling.

In the Kjeldahl method used for the determination of total nitrogen, the plant material was digested by 1.5 ml of concentrated sulphuric acid in the presence of anhydrous sodium sulphate and mercury catalyst ($^1/_4$ of a catalyst tablet as supplied by British Drug Houses Ltd.). The digest was transferred to a Markham still and after addition of 8 ml of 40% sodium hydroxide solution containing 0.44 g of sodium thiosulphate the ammonia was distilled off into 20 ml of 0.25% boric acid solution containing bromocresol green and methyl red. The ammonia was then determined by titration with $N/100$ $H_2SO_4$.

## RESULTS

### (a) *Growth of the seedlings*

Three weeks after transplanting it was quite noticeable that the growth of the seedlings was much better, the plants being larger and the leaves greener, in some pots than in others. These differences increased as the growing season progressed. One plant in pot $D_3$ showed abnormalities three weeks after transplanting, some of the leaves having died and turned brown although small new leaves had been produced; this plant subsequently died and the plants of this pot were therefore disregarded in the calculations.

### (b) *Stem lengths of seedlings*

The average stem length for each treatment was calculated and the values represent means for 16 seedlings for each treatment except in the case of the seedlings from the Chamaenerion caseinate treatment where the mean value is derived from measurements from 12 seedlings since one seedling in one of the Chamaenerion caseinate pots died half way through the growing period and the seedlings in this pot were excluded from further consideration.

TABLE 1

Mean stem heights of the birch seedlings from the various treatments

| Treatment . . . . . | A | B | C | D | E |
|---|---|---|---|---|---|
| Mean stem height of seedlings in cm . . . . . | 0.6 | 0.7 | 2.5 | 1.5 | 1.7 |

TABLE 2

Oven-dry weights, percentage nitrogen content and total nitrogen content of the leaves, stems and roots of the seedlings from each treatment and the root/shoot ratios of the seedlings from each treatment

| Treatment | Leaf dry weight in g | Leaf nitrogen content % of dry wt. | Total nitrogen content of leaves in mg | Root dry weight in g | Root nitrogen content % of dry weight | Total nitrogen content of roots in mg | Stem dry weight in g | Stem nitrogen content % of dry weight | Total nitrogen content of stems in mg | Total dry weight of seedlings in g | Total nitrogen content of seedlings in mg | Root/shoot dry weight) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.031 | 0.542 | 0.17 | 0.065 | 0.873 | 0.57 | 0.012 | 14.38 | 0.17 | 0.108 | 0.91 | 1.51 |
| B | 0.035 | 0.654 | 0.23 | 0.102 | 2.211 | 2.26 | 0.019 | 22.59 | 0.43 | 0.156 | 2.92 | 1.89 |
| C | 0.207 | 0.613 | 1.27 | 0.620 | 0.996 | 6.18 | 0.141 | 15.07 | 2.12 | 0.968 | 9.57 | 1.78 |
| D | 0.109 | 0.584 | 0.64 | 0.370 | 0.939 | 3.47 | 0.065 | 14.34 | 0.93 | 0.544 | 5.04 | 2.13 |
| E | 0.147 | 0.593 | 0.87 | 0.465 | 0.888 | 4.13 | 0.085 | 14.88 | 1.26 | 0.697 | 6.26 | 2.00 |

(c) *Oven-dry weights, percentage nitrogen content and total nitrogen content of the leaves, stems and roots of the seedlings from each treatment and the root /shoot ratios of the seedlings from each treatment*

The values given (Table 2) are average values for leaves, stems and roots per pot for each treatment. The mean values per pot are from four pots of seedlings except in case of treatment D where the mean value is from three pots of seedlings.

(d) *Dry weights and nitrogen contents of materials added to the pots in the various treatments*

Dry weights (constant weight at 80°C) of the complexes formed by the various leaf extracts with 0.30 g casein and their nitrogen contents *i.e.* the amount of nitrogen added to the various pots in the form of protein complex.

TABLE 3

Dry weights and nitrogen contents of the casein-leaf extractive complexes obtained from 0.3 g casein.

| Source of leaf extract . . . . . | *Calluna vulgaris* | *Circaea lutetiana* | *Chamaenerion angustifolium* |
|---|---|---|---|
| Dry weight of complex in g . . . . | 0.407 | 0.435 | 0.435 |
| Nitrogen content of complex in mg | 37.3 | 39.6 | 40.0 |
| Nitrogen content per g of complex in mg . . . . . | 91.7 | 91.1 | 92.0 |

The casein contained 131.5 mg nitrogen per g *i.e.* the 0.30 g of untreated casein added to a pot contained 39.5 mg nitrogen.

The Calluna raw humus, H-layer material, added at the rate of 1 g per pot contained 11.9 mg of nitrogen per g.

Average nitrogen content of 4 birch seeds (mean of three determinations) 0.066 mg.

## DISCUSSION

The results from a number of investigations into the influence of various root-environmental factors on the relative growth of roots and shoots of young plants have been considered by Curtis and Clark [7]. They conclude that as a general rule an increase in moisture or of available nitrogen will decrease relative root growth and increase relative shoot growth irrespective of whether the actual root growth is increased or decreased. The results quoted by Curtis and Clark for root/shoot ratios of plants grown in water

culture are of a different order, being approximately one tenth of those for plants grown in soil or sand, although it should be noted that the ratios for the culture solution plants are calculated on a dry-weight basis while for the plants grown in soil or sand they are on a fresh-weight basis. The root/shoot ratios of the birch seedlings in the experiments of Mikola [27] and Ingestad [18] are similar, except at the lowest levels of nitrogen supply, to those quoted by Curtis and Clark for barley plants grown in culture

TABLE 4

Root/shoot ratios obtained by various investigators for various plant species grown under a variety of conditions

| Author | Plant species used in experiment | Conditions of growth | Basis on which root/shoot ratio is expressed | Factors varied and corresponding root/shoot ratios (R/S) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Mikola [27] | *Betula pubescens* | Sand culture in flasks | Dry weight | Factor | Nitrogen per flask, mg | | | | |
| | | | | | 0.23 | 2.35 | 8.71 | | |
| | | | | R/S | 0.46 | 0.27 | 0.09 | | |
| Ingestad [18] | *Betula verrucosa* | Culture solution | Dry weight | Factor | Nitrogen supply in ppm | | | | |
| | | | | | 4.2 | 28 | 140 | 280 | 560 |
| | | | | R/S | 0.35 | 0.13 | 0.12 | 0.12 | 0.11 |
| Kozlowski [21] | Overcup oak (3-yr. old) | Soil | Dry weight | Factor | Shade | | Full light | | |
| | | | | R/S | 1.92 | | 2.08 | | |
| | Loblolly pine (3-yr. old) | Soil | Dry weight | R/S | 0.85 | | 1.22 | | |
| | Loblolly pine (2-yr. old) | Soil (in greenhouse) | Dry weight | R/S | 0.50 | | 0.63 | | |
| | Loblolly pine (2-yr. old) | Soil (outdoors) | Dry weight | R/S | 0.49 | | 0.70 | | |
| Curtis and Clark [7] | Barley | Culture solution | Dry weight | Factor | Nitrogen supply, ppm | | | | |
| | | | | | 40.5 | | 405 | | 810 |
| | | | | R/S | 0.169 | | 0.112 | | 0.105 |
| | Corn | Sand culture | Fresh weight | Factor | Percentage of water in sand | | | | |
| | | | | | 11 | 15 | 20 | 30 | 38 |
| | | | | R/S | 2.76 | 2.09 | 1.54 | 1.19 | 1.12 |
| | Carrot | Soil | Fresh weight | Factor | Nitrogen supply | | | | |
| | | | | | Low | | Medium | | High |
| | | | | R/S | 4.16 | | 2.45 | | 2.00 |

solution: Ingestad's seedlings were grown in culture solution and Mikola's, although grown in sand, were contained in glass flasks. On the other hand, however, the root/shoot ratios, on a dry-weight basis, of young oak and pine plants grown in soil (Kozlowski [21]) are on the whole closer to those quoted by Curtis and Clark for corn seedlings grown in sand and carrots grown in soil; all these are of a similar order to the root/shoot ratios of the birch seedlings grown in sand in the present experiment (Table 1).

In spite of these differences in the values for the root/shoot ratio in aqueous environments as compared with sand or soil, under both conditions, where the amount of nitrogen supplied is known, the evidence suggests that increases in the amount of nitrogen supplied results in a decrease in the root/shoot ratio. In the case of the birch seedlings in the present investigation, however, the root/shoot ratios do not seem to be related to the nitrogen content of the seedlings and certainly show no tendency to decrease in the seedlings of higher nitrogen content. This may perhaps be taken to be an indication that the concentration of available nitrogen was in no case sufficiently high to influence the root/shoot ratio although in no case, even where least nitrogen has been absorbed, is the root/shoot ratio very large.

However, if log shoot weight is plotted against log root weight for the values obtained in the experiments of Mikola [27] and Ingestad [18] and also for those of the present experiment it appears from the regressions, for the values from these various experiments, that the relative growth of roots and shoots is very similar for the birch seedlings from the various treatments in all three experiments (Fig. 1). In the case of Mikola's results the root and shoot dry weights have been multiplied by ten in order to accomodate them on the same graph as the results from the other experiments. It should be noted that in the case of the data from Ingestad's experiment the values for dry weight of root and shoot for the seedlings grown at the lowest nitrogen level are out of harmony with the corresponding values for the seedlings from the other treatments, even though the actual dry-matter production is much greater than the lower values of the present experiment and those of Mikola's experiment, and they were not used in the calculation of the regression. The actual concentrations of nitrogen at the lowest levels used were similar for Mikola's and Ingestad's experiments although the total amount of nitrogen available to the seedlings,

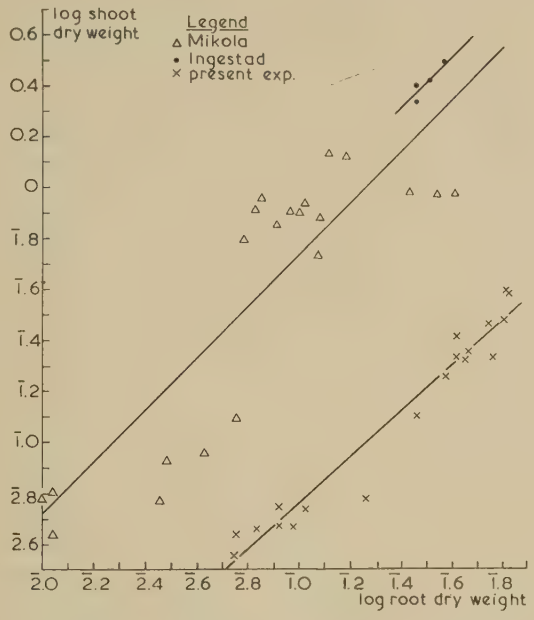


Fig. 1. Correlation between shoot and root dry weight of birch seedlings from various treatments from the investigations of Mikola [27] and Ingestad [18] and from the present experiment.
Mikola values for single seedlings multiplied by 10
Ingestad values per group of four seedlings
Present experiment values per pot of four seedlings.
Regression equations of the results

| | |
|---|---|
| Mikola | $\log_{10} y = 0.76 + 1.02 \log_{10} x$ |
| Ingestad | $\log_{10} y = 0.94 + 1.02 \log_{10} x$ |
| Present exp. | $\log_{10} y = -0.33 + 0.91 \log_{10} x$ |

in the much larger volume of solution in contact with the roots. was many times greater in Ingestads's experiment.

The marked differences in the dry weight of the birch seedlings from the pots of the various treatments in the present experiment is associated with considerable differences in the amounts of nitrogen absorbed by the plants even though very similar amounts of total nitrogen and other nutrients were added to each pot (Fig. 2).

The stems of the seedlings from all the treatments contained a relatively high concentration of nitrogen. This may have resulted from movement of nitrogen into the stems from the leaves before leaf fall for Tamm's [36] observations showed that the nitrogen content of birch leaves, expressed as a percentage of the dry weight,

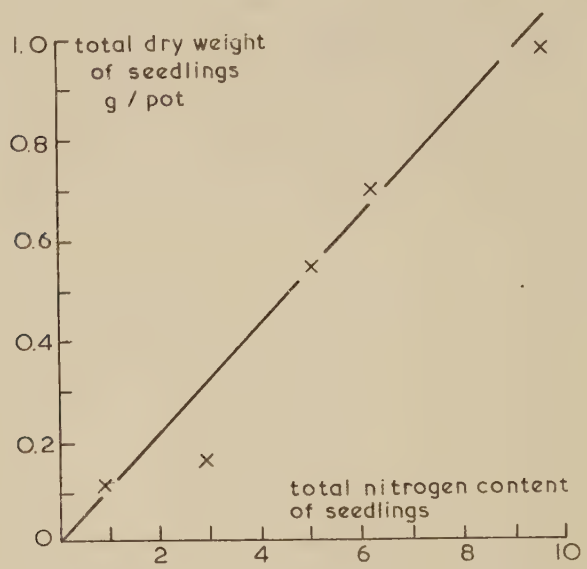


Fig. 2. Correlation between seedling dry weight (g) and seedling nitrogen content (mg per pot) of four seedlings, for the various treatments.
Regression $y = -0.004 + 0.108x$

decreased considerably at the time of leaf fall when the levels recorded (about 0.75%) were of a similar order to those found in the present experiment (about 0.6%).

Except in the case of seedlings grown in the presence of Calluna raw humus, where less nitrogen was added per pot initially than in the other treatments, as a source of nitrogen the amount of nitrogen absorbed by the seedlings from Calluna caseinate was considerably less than from the other sources of nitrogen. This is in agreement with a previous observation (Handley [14]) that the complex formed from gelatin and extractives from fresh Calluna material was solubilized more slowly by fungi than the complex formed from gelatin and extractives from fresh Chamaenerion leaves. Most nitrogen was absorbed by birch seedlings grown in the presence of the Circaea—casein complex; even more than by seedlings grown in the presence of untreated casein. Fenton [8] found that mineral nitrogen was rapidly released from casein added to a calcareous soil and it seems likely that under the conditions of the present experiment a considerable proportion of the nitrogen of the untreated casein

was released in the form of ammonia and, as there was no system in the sand to retain it, it was lost to the atmosphere before the root systems of the birch seedlings were large enough to absorb it.

If the relative amounts of nitrogen absorbed by the birch seedlings grown in the presence of Calluna, Chamaenerion and Circaea casein complexes reflect the relative resistance of these complexes to decomposition it would seem that, although similar amounts of casein give rise to similar amounts of complex containing similar amounts of nitrogen independent of the leaf extract used, there are considerable differences in the amounts of nitrogen released from the three casein complexes and in their resistance to decomposition. It is perhaps of considerable significance that the nitrogen contents of the seedlings from the Calluna raw humus and Calluna caseinate treatments (0.91 and 2.92 mg per pot respectively) are very nearly proportional to the amounts of nitrogen initially added to the pots (11.9 and 37.3 mg per pot respectively) for, if it can be assumed that all the nitrogen liberated in each pot was absorbed by the seedlings, this could be interpreted as indicating that the nitrogen of the Calluna raw humus and the nitrogen of the Calluna caseinate have similar properties and as supporting the contention that the residual leaf proteins of some species form similar complexes, following the death of the leaf, which remain almost unchanged or change only very slowly over considerable periods of time after falling on to the surface of some soils. Even in the case of Circaea caseinate apparently only approximately 25 per cent of the nitrogen was mobilized, the amounts being less in the case of the other caseinates, and one would like to know whether nitrogen continues to be mobilized at similar or different rates beyond this point. The presence of such systems in leaves would mean that residual leaf proteins in litter would be more resistant to decomposition in some plant species than in others. The formation of such complexes in leaves is likely to protect the walls of the cells in which they occur from enzymic degradation. In addition, the formation of such protein complexes will result in diminished rates of release of mobilized nitrogen, which can be utilized by soil animals, microorganisms and plants, from residual leaf proteins; it may also be associated with diminished rates of release of other inorganic plant nutrients which are not released until residual proteins are decomposed. Any scarcity of mobilized nitrogen resulting from such systems could

also be associated with delay in the decomposition of non-nitrogenous organic constituents of litter due to restriction of the growth and activities of micro-organisms concerned in the decomposition of these materials.

In the case of plant species giving rise to leaf protein complexes of this kind which are very resistant to decomposition it is to be expected that, under some conditions, at least the litter of such species will decompose so slowly that it will tend to accumulate and this may be expected to have a marked influence on soil processes. This is in fact the case with the species (*Calluna vulgaris*) producing the most resistant protein complex in the present experiment for it gives rise to the raw humus of Calluna heathland soils. It also seems feasible that as this very resistant material is reduced to finer and finer particles during successive ingestions by members of the soil fauna it will ultimately be in such a fine state of division that some of it will move down to the lower horizons of the soil, expecially when the mineral material is largely sand, under the influence of downward percolating water. *Chamaenerion angustifolium*, leaf extractives of which gave a protein complex apparently less resistant to decomposition than the protein complex formed by Calluna extractives, although colonizing disturbed or burned areas of raw humus also grows on mull soils, in fact according to Tamm [37] this species requires abundant supplies of nitrogen for growth, and does not seem to give rise to raw humus. On the other hand *Circaea lutetiana*, the species which gave the casein complex apparently least resistant to decomposition, is a plant which appears to prefer base-rich soils and which is almost certainly not associated with the formation of raw humus. In such cases the presence of residual leaf proteins in litter in the form of complexes having somewhat greater resistance to decomposition, compared with uncomplexed protein, would diminish the possibility of too rapid release of nitrogen which might occur if uncomplexed protein reached the soil in litter; loss of mobilized nitrogen from the soil system before it could be utilized by plant roots or micro-organisms would thereby be lessened. There seems to be agreement, therefore, between the field characteristics of these three plant species and the probable nitrogen mobilization characteristics of the residual leaf proteins of these species as deduced from the results of the birch seedling experiment.

Resistance to decomposition of leaf proteins due to the formation of complexes may be expected to influence the growth of animals dependent on such sources of nitrogen. In this respect it is probably significant that the biomass of the soil fauna is considerably smaller in the case of soils associated with raw humus and probably reaches the highest levels on soils of adequate depth and base status which receive regular heavy dressings of the dung of the herbivorous animals whose diet is likely to include a large proportion of plant material, *e.g.* grasses, whose protein is not likely to be in the form of resistant complexes.

Although it is sometimes considered that tannins in leaves may make them unpalatable to browsing animals, *e.g.* Wilkins *et. al.*[42], it does not seem to have been suggested that they may also render the leaf proteins, especially in fodder which has been dried, less digestible even though this would have an important effect on the fodder value of such leaves. Some herbivorous animals do eat plant material in which the protein-complex-forming system is present. Calluna heathland, with a vegetation often consisting almost entirely of *Calluna vulgaris* and associated with the formation of raw humus, has in many cases been maintained by periodic burning for the production of sheep over long periods of time. The sheep must derive at least some of the nitrogen for tissue production from the ingested Calluna material. Thomas [38] has considered the role of heather as a food for various animals and concluded that various non-carnivorous animals living on Calluna moorlands or heathlands consume more or less large quantities of heather. As far as sheep and cattle are concerned certain breeds seem more suited to this type of food material, or perhaps to the environmental conditions of the habitat, than others. It seems clear that sheep can grow satisfactorily when the vegetation contains a proportion of plants other than Calluna but whether sheep can grow satisfactorily on a diet of Calluna alone seems to be problematical. In spite of the longstanding and extensive practice of maintaining Calluna vegetation for the growth of sheep there have been only comparatively few attempts to study the digestion of Calluna fodder by animals. The earlier work, which has been reviewed by Armstrong and Thomas [1], apparently suffered from various shortcomings. The results of this earlier work usually indicate the digestibility of the crude protein of Calluna material as being of the order of 10 per cent which compares very unfavourably with the digestibility of the crude protein of hay which is usually given as of the order of 50 per cent.

In more recent times there have been further investigations into the digestion of heather by sheep. Peart [31] measured the body-weight increases, at frequent intervals between May and the end of August, of two similar groups of lambs, one group feeding on hill pasture which did not contain Calluna and the other on an equal area of adjacent hill pasture where Calluna was predominant over 90 per cent of the area. The number of sheep and

lambs on each area were similar and such that in the case of the pasture containing Calluna the lambs must have taken a considerable proportion of heather in their diet. There appears to have been very little difference in the daily weight gain per lamb for each monthly period or over the whole period on the two pastures and it is deduced that heather vegetation is as good as the non-heather pasture for lamb nutrition.

Thomas and his colleagues have made a number of studies on the problem of the digestion of heather by sheep. In one experiment (Armstrong and Thomas [1]) the lignin ratio technique was used to determine the digestibility coefficients for the various constituents of the fresh Calluna eaten by sheep tethered on heathland bearing only Calluna. This method gave values of the order of 40 per cent for the digestibility of the crude protein if the Calluna; these values are very considerably greater than those previously reported by continental workers but they would seem to give support to Peart's contention regarding the value of Calluna for the growth of lambs.

Armstrong and Thomas [1] and Thomas and Smith [39] have also used the metabolism crate method to assess the digestion of Calluna by sheep but in these experiments dried and not fresh heather was used. Heather was cut from 4-year-old and 10-year-old stands and immediately dried in a forced-draught oven at a temperature of 35–38°C. The leaf material was then readily separated by hand rubbing and the resulting material, which was considered to approximate sufficiently closely to the part of the Calluna plant eaten by sheep under normal grazing conditions, used for comparison of its digestibility with that of hay prepared in the traditional manner. When this Calluna material (from the 4-year-old stand) was fed alone to the sheep the daily food intake was very unsatisfactory; this was put down to unpalatability and dryness of the food although moistening did not improve matters. It is stated that it is known that sheep confined to heather under natural conditions will sicken of it in the course of a few weeks and that there is a good deal of evidence that sheep cannot subsist for more than a brief period on heather alone although there is no mention that food intake was inadequate in the experiment with sheep tethered on heather; in fact it is suggested that under such conditions the already considerable digestion of crude protein increases with time. The experience of these authors was that sheep in metabolism crates could not be kept on a diet consisting wholly of heather (dried) for more than 5 or 6 days without serious loss of condition. However, it is of considerable interest that, according to the results of these investigations, of the heather eaten a considerable proportion of the non-nitrogenous constituents was digested (digestibility coefficient of 40.7 compared with 53.8 for the non-nitrogenous fraction of hay) whereas the crude protein was apparently completely indigestible compared with a digestibility coefficient of 45.3 for the crude protein of hay. This is rather surprising as one would expect a lack of digestible protein in the diet to reduce considerably the activities of the rumen micro-organisms which break down carbohydrate material although if conditions are equivalent to starvation as far as nitrogen is concerned, then non-protein nitrogen could be supplied to the rumen from the animal's own body, as suggested by Chalmers and Synge [5], and this

may be a source of the serious loss of condition in the animals. Further experiments were carried out in which mixtures of various proportions of hay and heather, both having similar initial crude protein contents, were fed to sheep in metabolism crates. From the results of these investigations it is deduced that the crude protein and crude fibre have digestibility coefficients in the case of heather (13 to 23 for crude protein and 30 for crude fibre) which are rather less than half of the corresponding coefficients for hay (41 to 49 for crude protein and 63 for crude fibre). The only well-marked difference between the younger and older heather is that the crude protein digestibility of the older heather is about 30 per cent less than that of the younger heather. It is concluded that as a source of protein even young heather is poor.

Although there are considerable uncertainties in the interpretation of the results of experiments of this kind the results for the digestibility of heather protein from these various investigations show such wide variation that certain points of interest arise. When Calluna was eaten in a fresh state either by lams or tethered sheep, the digestibility of the crude protein seemed to be little less than that of hay. Heather which has been dried at a very moderate temperature and mixed with hay for feeding seems to have a crude protein digestibility which is $^1/_3$ to $^1/_2$ that of the crude protein of fresh Calluna, that from a 10-year-old Calluna stand being apparently less than that from a 4-year-old stand and the lowest values were very similar to those obtained by earlier continental workers. When dried heather only is fed to sheep, the crude protein seems to be completely indigestible even though Thomas and Smith [39] state that no evidence could be obtained that drying at the temperatures used had any appreciable effect on digestibility. If the protein in the heather were present as protein alone and not combined with other materials it might even be expected that a moderate degree of heating would increase its digestibility.

There are therefore indications that the digestibility of the crude protein of fresh Calluna is greater than that of dried Calluna. If this be considered in terms of tannin-like protein-precipitating materials in leaves it is clear that when fresh Calluna is ingested and complex formed by the leaf protein, this will not undergo drying whilst in the alimentary tract of the sheep and for this reason alone it is to be expected that the protein would remain more digestible than if the heather is fed after being dried although it might be expected that the tannin-like materials would inactivate the proteolytic and other enzymes of the rumen micro-organisms. This would be in agreement with observations (Handley [14]) that air-dried gelatin–Calluna complex seems to be more resistant to solubilization by fungi than similar complexes which have not been allowed to dry out. In addition it is likely that little autoxidation and polymerization of the protein-precipitating substances will take place, either before or after union with leaf protein, during its passage through the alimentary tract although some oxidation may take place during rumination. Fresh heather kept submerged in water in the presence of chloroform and in the absence of oxygen retains a greenish yellow colour but if exposed to the air under the same conditions the surface

layer rapidly becomes dark brown .Storage of an aqueous extract of fresh Calluna shoot material results in the loss of its protein-precipitating capacity and the deposition of material which is probably a polymerized and oxidized form of the protein-precipitating material, perhaps also united with other leaf polyphenolic materials. It is envisaged that processes somewhat similar to those described by Hathway and Seakins [15] and Roux [33] have taken place.

Absence of drying, oxidation and polymerization may all be expected to reduce the potential indigestibility of the protein complex. It is possible that in the preparation of the dried heather for the sheep feeding experiments, various degrees of oxidation, polymerization and union with other leaf polyphenols may have taken place in the various samples, depending on the rapidity of drying and length of time of storage before use. This variability may be the basis of the differences between the apparent digestibilities of the crude protein of dried heather when fed alone and when fed mixed with hay; it may also be that hay has some synergistic action on the digestion of the heather protein. It is of considerable interest that the crude protein digestibilities of the similarly prepared samples of dried heather from the 10- and 4-year-old stands showed marked differences whereas the digestibility coefficients of the crude fibre in the two cases is very similar but about half the value of that for hay. The heather from the older stand may well contain, at the time of collection, a greater proportion of leaf material which has already died naturally but which has not fallen to the ground and it is to be expected that the protein complex of this material will be fully dried, oxidized and polymerized and therefore have maximum resistance to digestion which could account for the apparently lower digestibility of the crude protein of the heather from the older stand. On the other hand, when the cells of the heather leaf die the protein complex formed will protect a proportion of the cell wall material from the action of enzymes and the amount of wall thus protected is not likely to be increased by drying, oxidation and polymerization of the protein complex except in so far as it lowers the speed of any removal of the protecting protein complex during passage through the alimentary tract of the sheep. This may well be the basis of the lack of difference between the crude-fibre digestibilities of the heather from the 10- and 4-year-old stands. By contrast the crude protein of hay, which has undergone drying and storage during its preparation, is digestible to a very considerable degree.

Sheep were also used in investigations by Folgado [9] into the digestibility of the fresh leaves of *Ulmus carpinifolia* (Gled.) and *Populus nigra* (L.) by the chromogen-indicator method. Expressed as a percentage of the dry matter, the leaves of elm contained 15.74 per cent of crude protein whilst those of poplar contained 8.39 per cent; the animals apparently preferred the poplar leaves. It is concluded that 52.9 per cent of the elm leaf protein was digested, compared with 64.3 per cent of the poplar leaf protein, the corresponding figures for crude fibre being 19.6 and 60.4 per cent. In terms of fodder units these leaf materials are said to be comparable with meadow hay of average quality. If it is permissible to compare these results with

those obtained for fresh Calluna the protein digestibility is certainly greater than the value of 40 per cent obtained by Armstrong and Thomas [1], using tethered sheep, and much greater still than the values for dried Calluna fodder.

The digestibility of two other tree leaf fodders, *i.e.* those of *Fraxinus ornus* and *Quercus* spp., has been investigated by Vukavić and Maksimović [40] using sheep and methods similar to those of Armstrong and Thomas [1] and Thomas and Smith [39]. The fodder included small twigs such as would normally be eaten by the grazing animal. Material from the two kinds of tree was collected separately each month and stored, presumably in an air-dry condition, until the following spring when they were used in feeding experiments. The samples of oak fodder were fed without admixture with other materials whereas in the case of the samples of ash fodder a ration of ash fodder, amounting to half the total amount of oak fodder fed daily to each sheep, was fed to each sheep in the evening and a somewhat smaller weight of oats each morning. The digestibility of the ash fodder was determined from chemical analyses of the food and faeces and the digestibility characteristics of the oats (Table 5).

TABLE 5

Composition and digestibility of oak and ash fodder and oats from Vukavić and Maksimović [40]

| Type of fodder | Crude protein | | Crude fibre | |
|---|---|---|---|---|
| | Content as a percentage of dry matter | Digestibility coefficient | Content as a percentage of dry matter | Digestibility coefficient |
| *Oak* | | | | |
| July fodder . . . . | 13.58 | 32.74 | 25.34 | 43.49 |
| August fodder . . . | 12.30 | 19.48 | 26.15 | 35.76 |
| *Ash* | | | | |
| July fodder . . . . | 9.63 | 43.11 | 22.72 | 45.92 |
| August fodder . . . | 11.11 | 35.30 | 21.19 | 44.80 |
| September fodder . . | 9.87 | 19.33 | 26.06 | 40.80 |
| *Oats* . . . . . | 13.35 | 77.0 | 12.82 | 24.10 |

The sheep used to assess the digestibility of the August sample of oak fodder were then used, on the day following the completion of this test, for the July sample and it seems possible that the August sample test may have conditioned both the animal and the rumen microflora to oak fodder thereby upsetting the comparison of the digestibility of the two samples although all the sheep apparently had a preliminary period of 4 weeks in which to get used to the experimental conditions and leaf fodder. The sheep which had been used for the August and July samples of oak fodder were immediately

used for the assessment of the August sample of ash fodder and the relative values for the digestibility of this sample may be too high. It is possible that the digestion of the samples of ash fodder has been influenced by the use of oats. The body weight of all the sheep decreased slightly during the course of the experiments.

If it is permissible to compare these results with those obtained for dried Calluna fodder, the crude protein of the oak fodder is apparently much more digestible than that of dried Calluna fodder when fed alone, and rather more digestible, especially in the case of the July sample of oak fodder, than that of dried Calluna when fed with hay. The crude protein of the July sample of ash fodder when fed with oats had a digestibility equal to that of the hay in the experiments of Armstrong and Thomas [1] and Thomas and Smith [39] when mixtures of hay and dried Calluna were used, and greater than that of the samples of oak fodder.

It is of considerable interest that although there is no marked change in the crude-protein content of oak and ash fodder during July to September the digestibility of the crude protein shows a marked fall. This parallels the probable increase in polyphenolic materials of various kinds in the leaves as the growing season advances.

These various observations therefore suggest that the digestibility of leaf protein differs for different plant species and in some cases may become extremely resistant to digestion if the leaf undergoes drying in air as it does when it dries naturally and becomes litter. Such resistant material would be the form in which the residual leaf protein in the litter of such species would be received by the soil animals and micro-organisms.

The contention that the protein precipitating substances of leaves are of fundamental importance in soil processes, especially raw-humus formation, and plant growth is therefore strongly supported by experimental approaches from very different directions.

It is well known that a number of tree species, more especially the conifers, are associated with the formation of raw humus under some conditions and it is also known (Handley [14]) that such tree species contain protein-precipitating substances in their leaves and it should not be overlooked that the leaves of some plant species are notable for their high content of tannins. If, as seems probable, such substances are closely connected with the development of resistance to decomposition by residual leaf proteins it is of considerable importance from the point of view of soil processes, plant nutrition and perhaps also animal nutrition under some circum-

stances to understand the factors determining the variable resistance to breakdown of the leaf protein complexes formed by different plant species and also the conditions which facilitate or impede the breakdown of these protein complexes. Delay in the mobilization of the nitrogen of protein complexes is also likely to hold up the breakdown of non-nitrogenous organic constituents of plant litter by hindering the growth and activities of micro-organisms and also by hindering the development of the soil fauna. Reduction of soil faunal activity would result in the litter remaining on the soil surface in an uncomminuted form for a longer time and therefore more subject to drying out than if it had been injested by the soil animals and subsequently deposited at or below the surface of the soil where moisture conditions are usually more favourable for the activities of micro-organisms.

The experiments with birch seedlings indicate that when placed under similar conditions there are marked differences in the behaviour of protein complexes prepared from extractives from the leaves of different plant species. It is therefore of importance to enquire into the possible reasons for these differences.

The leaves of certain plants are used as sources of commercial tanning materials and such substances could unite with residual leaf proteins on the death of the leaf and therefore the resistance to decomposition of leathers prepared by the use of different vegetable tanning materials seems to provide a starting point for such an enquiry. Gustavson [12] found that although the native collagen of calf skin is very resistant to the action of trypsin the native collagen of fishskin, and especially that of cod (*Gadus morrhua*), is very easily digested by trypsin. The prepared cod-skin collagen was tanned to completion with a variety of tanning materials including Mimosa bark, tannic acid, Gambier and Sumac extracts and then washed until free from soluble materials. Digestion with trypsin, the solution being maintained at a reaction of pH 7.8 by $CaCO_3$, was carried out at a temperature not exceeding 30°C and the amount of collagen digested was determined by estimating the total nitrogen in the filtrates by the Kjeldahl method. (Table 6).

In the birch-seedling experiment the casein complexes absorbed protein-precipitating materials from the leaf extracts to the extent of 36 to 45 per cent of the weight of casein. These are of the same order as the percentage of tannin material fixed by eel-skin collagen and very similar to the values for the final degree of tannage quoted by Phillips [32] for cow hide tanned by various vegetable tannins. Much higher values for tannin fixation, of the order of 150 per cent, by gelatin are quoted by Gustavson [13] although the amount of tannin fixed is probably influenced to some extent by the amount and concentration of tannin used.

TABLE 6

The composition of the tanned fishskin collagen preparations from Gustavson [13]

| Tanning material | Codfish collagen | Eel collagen |
|---|---|---|
| | Percentage fixed tannin on collagen basis | Percentage fixed tannin on collagen basis |
| Mimosa bark . . . . . | 28.5 | 57.7 |
| Tannic acid . . . . . | 28.1 | – |
| Gambier extract . . . . . | 24.5 | – |
| Sumac extract . . . . . | 20.7 | 32.4 |

Although in Gustavson's fishskin-collagen experiments tannin was liberated from the tanned collagen during incubation with trypsin and this resulted in a marked loss (from 60 to 80 per cent) in the activity of the trypsin, the percentages of collagen digested by the trypsin in the case of the differently tanned fishskins is of considerable interest (Table 7).

TABLE 7

The digestion of fishskin collagen tanned by various tannins (from Gustavson [12]) (as percentage of collagen digested by trypsin)

| Tanning material | Codfish skin | Eel skin |
|---|---|---|
| Mimosa bark . . . . . | 4.9 | 1 |
| Tannic acid . . . . . | 11.7 | – |
| Gambier extract . . . . . | 5.0 | – |
| Sumac extract . . . . . | 14.0 | 4 |

Whilst the vegetable tanned collagen was partly stripped of tanning agents in the slightly alkaline digestion solution, there was no noticeable effect on either chrome or formaldehyde tanned cod-skin collagen under similar conditions and tryptic solution showed no loss of activity after contact with these latter materials.

A little later, Lipsitz, Kremen, and Loller (cited by Gustavson [13]) investigated the effect of trypsin on calf-skin leathers which had been produced by the use of vegetable tannins. In the case of leather prepared by the use of Mimosa and Quebracho extracts only 5 per cent of the original collagen was digested whereas when Chestnut wood extract was used from 40 to 60 per cent of the original collagen went into solution.

Commercial vegetable tanning materials are frequently separated into two groups, the hydrosylable tannins which are characterized by ester linkages and the condensed tannins which are characterized by linkages between the phenolic nuclei which are less readily broken than the ester links of the hydrolysable tannins. It would seem to be of importance that of the tanning materials used by Gustavson [12] and by Lipsitz *et al.*, those which imparted least resistance to digestion of the collagen by trypsin, *i.e.* tannic acid, Chestnut wood, and Sumac extract, are generally considered

to belong to the hydrolysable group of tannins whilst those which imparted most resistance, *i.e.* Mimosa bark, Gambier and Quebracho extracts, are included in the condensed tannins. The Sumac extract was almost certainly obtained from leaves whilst the Gambier extract was likely to have been obtained from the leaves and twigs of *Uncaria gambier*, the remaining tanning materials being obtained from bark or wood. Hathway and Seakins [15] extracted harvested leaves of *Uncaria gambier* and obtained a tanning material (hide powder tested) which contained only a single phenolic component which remained at the origin in two dimensional chromatograms; this was considered to be an oxidative polymerization product from catechin *i.e.* a condensed tannin which, it is suggested, may only arise on the death of the leaf tissues. Unfortunately there seems to be no information available regarding the influence of the litter of these two plants (*Rhus* spp. and *Uncaria gambier*) on the humus form of the soil on which they grow.

The leaves of a number of plants in various regions of the world are used in the manufacture of leather, the leaves of other plants have been used for this purpose in the past, chiefly in north temperate regions, and the leaves of yet other plants, although not used in the tanning of hides, are said to contain tannins. Information concerning the humus form associated with these plants appears to be available in comparatively few cases and in very few cases does anything appear to be known concerning the nature of the tannin (Table 8).

TABLE 8

Plant species whose leaves have been used for tanning or contain tannin (from Howes [17]) and their probable associated humus types

| Probable humus type with which the plant species is associated on other than base-rich soils | Plant species |
|---|---|
| Mull | *Fraxinus execlsior* – tannin said to be present in leaves. |
| Mull | *Spiraea* (*Filipendula*) *ulmaria* – leaves said to have been used formerly for tanning and dyeing in Iceland |
| Mull | *Carpinus betulus* – leaves said to contain ellagitannin |
| Mull | *Castanea sativa* – leaves contain tannin |
| Raw humus | *Calluna vulgaris* – said to have been used for tanning in the 18th century |
| Raw humus | *Rhododendron ferrugineum* – leaves used for tanning in Piedmont |
| Raw humus | *Vaccinium myrtillus* and *Vaccinium vitis idaea* – both used in small scale tanning in Europe |
| Raw humus | *Taxus baccata* – leaves as well as other parts of the plant contain tannin |
| Raw humus | *Picea abies* – needles contain about 5% of tannin |
| Raw humus | *Pinus* spp. – needles contain only relatively small quantities of tannin |

White [41] states that the bark, wood, leaves, and twigs of *Castanea* spp. contain hydrolysable tannin and according to Howes [17] the leaves of *Carpinus betulus* contain ellagitannin which is also a hydrolysable tannin. It is perhaps of significance that the litter of both these species, although taking some time to disappear on some soils, does not seem to give rise to raw humus. If this is related to the observations that collagen tanned with hydrolysable tannins is less resistant to decomposition than when tanned with condensed tannins then, in connection with the hypothesis that the formation of raw humus is associated with the presence of protein complexes in litter, one might expect to find condensed tannins or their precursors in the leaves of species associated with the formation of raw humus.

The leafy shoots of *Calluna vulgaris* contain a gelatin-precipitating substance which appears to contain leucocyanidin as part of its molecule; it has not been possible to demonstrate that the molecule contains carbohydrate (Love and Brown [22]) and this substance may well prove to be a condensed tannin.

It has not been possible to find any information regarding the nature of the tannins which, from their use as tanning agents, would seem to be present in the leaves of other species which are associated with the formation of raw humus. Although the bark, wood, leaves and twigs of *Castanea* spp. contain hydrolysable tannins and the tannins of spruce bark are condensed tannins (White [41]) it cannot be deduced that any tannins in the needles of spruce will also be condensed tannins for it has been shown that in some species different tissues of the same plant contain different types of tannin. Thus whilst the bark of *Quercus* spp. may contain condensed tannins the wood may contain hydrolysable tannins; similarly, although the tannins of Quebracho (from the wood of *Schinopsis* spp.) belong to the condensed group King and White [20] could find no phenolic nuclei which are considered to be characteristic of condensed tannins in extracts of the fresh leaves of various *Schinopsis* spp. and the leaves of these species seem to contain only hydrolysable tannins. It is perhaps of interest that the *Rhus* spp. whose leaves contain hydrolysable tannins, also belong to the Anacardiaceae.

The simpler phenolic materials are considerably easier to investigate than tannins and many different kinds have been found in the tissues, including leaves, of many plant species. Comparatively little is known concerning the role these simpler reactive phenols play in the formation of tannins and it is unwise to deduce much regarding the nature of tannins in leaves from the simpler polyphenols they may contain.

In the case of *Circaea lutetiana* and *Chamaenerion angustifolium* there seems to be little information available concerning the phenolic constituents of the leaves; aqueous extracts of the leaves,

which actively precipitate gelatin, have been observed to have the following properties (Table 9).

TABLE 9

Some properties of extracts of the fresh leaves of *Calluna vulgaris*, *Chamaenerion angustifolium* and *Circaea lutetiana*

| Treatment | *Calluna vulgaris* | *Circaea lutetiana* | *Chamaenerion angustifolium* |
|---|---|---|---|
| Storage of extract in presence of chloroform and air. | Soon deposits a considerable amount of reddish brown material, the supernatent becoming lighter in colour and losing its power to precipitate gelatin.* | After up to ten years only a small amount of light-coloured material is deposited and the extract retains its power to precipitate gelatin apparently undiminished. | Similar to Circaea extract. |
| Addition of ferric chloride solution | Dark greenish brown colour | Deep blue-back colour | Deep blue-black colour |
| Heating with hydrochloric acid | Extract becomes a deep red colour and phlobaphene is deposited. | No change in colour | No change in colour |
| Stiasny reaction. | Immediate very bulky flocculent precipitate in the cold which aggregates somewhat on heating. | Quite clear even on heating for some time but on cooling a light cream coloured powdery deposit settles out and the fluid is slightly turbid. | Quite clear even on heating for some time. On cooling a slight turbidity appears especially at the surface exposed to the air. |

* Raspberry leaf extract was found to give an increasing amount of sediment (Cadman [4]) and to have lost 90% of its virus-inhibitory activity during storage for 5 months although it is not stated whether any precautions were taken to prevent the growth of micro-organisms.

As far as it goes this evidence does not suggest that the Circaea and Chamaenerion extracts contain phenolic materials which are considered to be characteristic of condensed tannins; indeed, if anything, it suggests that any tannins which may be present are more likely to be of the hydrolysable type. This is perhaps supported by the finding of Bate-Smith [2] that ellagic acid appears to be

present in the acid hydrolysed leaf extracts from members of the genus Circaea which like Chamaenerion is included in the Onagraceae.

If it should prove that Calluna leaves contain condensed tannins whilst Circaea and Chamaenerion leaves contain hydrolysable tannins then, in view of the work of Gustavson [12] and Lipsitz *et al.* (Gustavson [13]) on the relative resistance to the action of trypsin of collagen tanned with condensed and hydrolysable tannins, one would expect protein complexes formed by *Calluna* extract to be more resistant to decomposition than those formed by extracts of the leaves of *Circaea* and *Chamaenerion*: if the nitrogen absorbed by the birch seedlings represents the relative resistance to decomposition of the various casein complexes then this seems to be the case. It also supports the hypothesis that an important factor in raw-humus formation is the resistance to decomposition of complexes formed between leaf constituents and residual leaf proteins on the death of the leaf.

The leaves of *Uncaria gambier* and *Eucalyptus redunca* are said to contain condensed tannins but the humus forming properties of the leaves of these species do not seem to have been investigated; much further work on the nature of the tannins of the leaves of various species is required before it is known whether a particular type of leaf tannin, e.g. condensed tannin, is associated with the formation of raw humus.

It is probable that different plants will contain a considerable variety of tannins and other phenolic materials in their cells and that these will give rise in turn to many different kinds of leaf protein complexes thereby imparting to the residual leaf proteins in different plant species a wide range of resistance to decomposition. There could well be a considerable range of degree of resistance within a single group of tannins, *e.g.* the hydrolysable tannins, and the different rates of supply of nitrogen from the Circaea— and Chamaenerion—casein complexes may be a case of this. It also seems probable that a larger number of differing phenolic nuclei will be found to be concerned in the formation of tannins than is usually considered to be the case at present, this could well increase the number of differing degrees of resistance to breakdown of any leaf protein complexes they may form.

In view of the apparent importance of leaf protein—polyphenol

complexes in the formation of raw humus, it is desirable to consider what conditions are likely to promote or hinder the breakdown of such complexes.

Archeological investigations have provided a certain amount of information regarding the persistence of animal protein which has been tanned. Ancient leather which has remained under waterlogged conditions, *e.g.* at the bottom of filled-in wells, appears to be well preserved. Similar material which has not remained under conditions of waterlogging has generally tended to disappear except when it has remained in extremly dry conditions or in close proximity to bronze objects.

Remains of hide, dating from Neolithic times onwards, which has been treated in one way or another have been found in various places. Stokar [34] discusses some of these finds, dating from 3,000 B.C. to 600 A.D., and although it is difficult to ascertain the type of tanning materials used, it seems clear from analyses that a variety of materials must have been used. Stokar states that the chemical characteristics of the soil in which the leather has been preserved constitute one of the chief difficulties in the determination of the tanning agents used; he considers that in the case of preserved leather from some moorlands the humic acids of the moorland have had a tanning effect; he also mentions Bronze Age leather preserved in the presence of bronze objects when the reaction of the soil was smaller than pH 6.4. He quotes the most interesting case of a leather sheath and carrying belt for a stone dagger, of the Neolithic period, found at Wiepenkathen. The sheath and belt, considered to have been made from three leathers tanned in different ways, had been preserved under identical soil conditions, unfortunately not stated, and the observations suggest that the best preserved leather had been vegetable tanned.

The preservation of skins and leather has also been considered by Gansser [11] and he has expressed the opinion that bark-tanned leather tends to be preserved under conditions of waterlogging, citing bark-tanned Roman leather which has kept very well in clay soil for 1900 years, or under dry conditions but that it will undergo decomposition and even complete dissolution in damp sandy soils or rich earths. He considers the latter to be the reason for the rare occurrence and bad state of preservation of leather found in European burial sites.

Two recent finds in France dating from more recent times, about the 8th century, are of interest. France-Lanord [10] has investigated a piece of preserved leather belt from which the stitches had disappeared, a small bag of goat-skin leather, hair and fabric of vegetable fibre and wool found alongside a bronze buckle. The remainder of the belt had apparently decomposed and disappeared from the magma found in a crumbling limestone sarcophagus. This contrasts with leather footwear, apparently well preserved and of the same period, found in another sarcophagus near Paris. It is not easy to suggest, from the account of these finds, the possible cause of the preservation of the leather, apparently in the absence of metal objects, in

the one case whereas in the other only those parts of the leather in contact with a bronze buckle were preserved. It seems just possible, apart from any difference in tanning materials used, that the environmental conditions have not been the same in both cases for the whole time since burial. The belt may have been in contact with calcareous material, perhaps moist, for a more or less long time which may have resulted in some detanning and microbiological decomposition whereas the footwear may have been protected from such conditions as their preservation seems to be considered a unique occurrence.

There have also been remarkable finds of preserved protein, under conditions which would seem to have been neither dry nor waterlogged, in Bronze Age barrows in Denmark [30]. Oak-log coffins containing excellently preserved garments of wool and coarser hairs, leather sandals and belts, cow skins and objects of wood and horn have been found in the barrows beneath the considerable mound of earth. Although the coffins generally contained bronze objects it seems to be considered that the tannin of the oakwood of the coffins has preserved these materials over this long period of time. The results of some recent [22] excavations at Crichel Down, Dorset, of crouched burials of the Beaker folk, when a wooden object of unknown use and a lingering trace of raw hide or leather thong were found along with the skeleton beneath a long barrow 7 feet high, contrast with the Danish finds. Both these burials took place at approximately similar times and apparently have since been under somewhat similar conditions apart from the presence of oak coffins and bronze objects in the case of the Danish burials. It seems possible that garments or wrappings were originally present in the Crichel Down burials but that these have long since disappeared completely and only traces of rawhide or leather remain. This may support the suggestion that the oak-wood tannins are responsible for the preservation of the Danish finds although the preservation of the coffins themselves and the wooden object at Crichel Down tends to suggest that conditions have not been very favourable for some kinds of microbiological activity at least, for dead roots would be expected to decay under such conditions at such depths although there is perhaps the likelihood of there being more direct connection with the atmosphere in the case of decaying roots.

The effect of micro-organisms on vegetable tanned hide and the use of leather as a fertilizer have been considered previously (Handley [14]). With one exception, in which the identity of the fungus is unknown, mycological action did not seem to cause significant change in the properties of vegetable tanned hide and, unless extensively detanned, leather does not seem to have appreciable fertilizer value for short rotation crops. Gansser [11], however, states that crushed leather is successfully used as a fertilizer on tobacco plantations in the U.S.A. but he does not indicate whether it is vegetable tanned hide or whether it is detanned before use; he also states that old shoes buried in the soil will be pierced by roots within a few years.

There is therefore evidence that protein combined with vegetable tannin may be preserved for very long periods of time under a variety of conditions or it may disappear. Such observations, however, do not give a great deal of help at the moment in understanding the factors concerned in the decomposition of vegetable tanned protein.

Little seems to be known of the processes concerned in the decay of leather and how far this is brought about by or associated with purely chemical processes and how far it can be brought about by biological agencies. Phillips [32] has discussed the rotting of vegetable tanned hide due to sulphuric acid; when leather completely disintegrates under such conditions 50 per cent of the total nitrogen becomes soluble but such a process is hardly likely to be related to that principally concerned in the decomposition of protein complexes in soils if only because rotting of the leather apparently did not take place unless the reaction of the aqueous extract of the leather was more acid than pH 3.0.

Vegetable tannins are considered to protect proteins from the action of enzymes because they interfere with the necessary contact between the enzyme and the protein molecules, it may therefore be expected that any process which results in removal of tannin from combination with protein will facilitate decomposition of the protein.

When a fibrous protein is treated with solutions containing a sufficient amount of tannin part of the tannin is fixed by the protein molecules and further tannin will be precipitated within the hide structure without contributing to the actual tannage. If after being washed free from uncombined tannin the tanned fibrous collagen is treated with water-miscible organic solvents and water, *e.g.* a mixture of acetone and water, some of the tannin will be stripped from the collagen whilst some will be almost irremovable even though the two fractions appear to be one and the same substance. It does not seem possible to remove the tannin completely by this method, indeed a high degree of tannage with quebracho has been obtained in the presence of acetone by Roddy (quoted by Phillips [32]). The degree of stripping does not seem to be related to the kind of tannin but the smaller the amount of tannin present the more firmly does it seem to be bound.

Fixed vegetable tannin can also be removed from fibrous collagen by treatment with concentrated solutions of urea and in this case the degree of stripping is markedly influenced by the kind of tannin. This treatment has little effect when condensed tannins have been used whereas hydrolysable tannins are largely stripped from the collagen, which may even be gelatinized.

In experiments with completely vegetable tanned fishskin Gustavson [12] considered that the alkaline reaction (pH 7.8), due to the $CaCO_3$ used to maintain a suitable reaction for the activity of the trypsin, resulted in stripping of fixed tannin from the collagen but he does not record whether there was any difference in the amounts of condensed tannins removed compared with the amounts of hydrolysable tannins removed.

There are indications, some of which have been mentioned by White [41], that some non-fibrous proteins, such as active virus particles, serum proteins and some enzymes, unite with tannins or other polyphenolic substances, including some present in leaves, giving water-insoluble complexes from which the protein can be regenerated in its former active state and having its former physicochemical characteristics, which would seem to indicate almost if not complete stripping of the tannin to have taken place, by various treatments such as the addition of other proteins, *e.g.* gelatin, or caffeïne (Mejbaum-Katzenellenbogen *et al.*[25]) to the system. Although the identity of the polyphenolic materials concerned is not indicated the regeneration of active protein suggests that in these cases the protein-polyphenol complex is more easily and more completely dissociated than is the case with the better known fibrous protein-tannin complexes. Whether this is due to the proteins having different characteristics or to some properties of the polyphenols concerned or to some other factor is not certain. The investigations of Cadman [4] suggest that while the particles of some plant virus diseases form complexes in vitro with leaf polyphenols from which active virus can be regenerated, by dilution of the suspension of complex with water or making the system less acid, the particles of other plant virus diseases cannot be regenerated, by the same means, from complexes formed with leaf polyphenols of the same kind. It is perhaps of significance from the point of view of the role of leaf protein—tannin complexes in litter decomposition that in those cases where active protein was regenerated from complexes which had been formed with leaf constituents the litter of the plant species concerned, *e.g.* strawberry, raspberry *etc.*, is not considered to give rise to raw humus.

Tannin which is not subsequently removed by aqueous acetone is also fixed by globular proteins, for example Danielsson (quoted by Gustavson [13]) obtained complexes, which resisted the action of acetone water (80/20), in which 100 g of the protein fixed 55 g of tannic acid. The amount of tannin fixed does not seem to be significantly affected by the reaction of the system so long as it is more acid than the isoelectric point of the protein. The isoelectric point of the proteins of many leaves falls in the range pH 4 to 5 (Lugg [23]) and the reaction of aqueous suspensions or extracts of fresh leaves and freshly fallen litter of many plant species lies close to the same pH range (see references cited by Handley [14]), so that on the death of such leaves it may be expected that there will be optimum fixation of any tannins which may be present in the leaf.

It is of interest that vegetable tanned leathers prepared from limed collagen, which has an isoelectric point in the region of pH 5, show maximum stability at a reaction of pH 5 (Gustavson [13]) When such protein–tannin systems are placed under less acid conditions they may be expected to become less stable although it must be remembered that Danielsson (quoted by Gustavson [13]) apparently obtained unprecipitated complexes, which were precipitated at less alkaline reactions, of globular protein and tannic acid at a reaction as alkaline as pH 8. Therefore although leaf protein–tannin complexes present in litter may be expected to become less stable the less

acid the soil environment in which they are placed, it is very unlikely that they will be completely detanned due to the soil alone even in the most calcareous soils. The addition of lime, and possibly other materials, in quantities sufficient to produce an appreciably less acid reaction, *e.g.* in acid raw humus layers, may be expected to decrease the stability of any leaf protein–tannin complexes present in a soil although the possiblity that the Ca-ion as such may play an important role in the biological decomposition of these substances should not be overlooked. Some detanning of litter protein complexes may also be expected from the circumneutral reactions which seem to occur in litter in moist conditions soon after it falls from the plant although the less acid reactions are perhaps less likely to occur in the case of those leaves which might be expected to give rise to the most resistant complexes (Handley [14]). Detanning of leaf protein–tannin complexes may also occur to some extent after ingestion of the leaf material by ruminants for the saliva, *e.g.* in sheep which secrete very large volumes of saliva, is slightly alkaline (Chalmers and Synge [5]). Appreciable concentrations of ammonia, associated with the breakdown of proteins, may apparently occur in the rumen of sheep during grazing (Mc Donald [24]) and it is conceivable that a sufficiently alkaline reaction might occur locally which would lead to the partial detanning of protein—tannin complexes in the fodder; this would be especially likely to occur in mixed diets where part of the fodder contains uncomplexed plant protein and may be part of the basis of the apparent increase in digestibility of the nitrogen of Calluna fodder when fed with a proportion of hay. Proteolytic enzymes may be inactivated by the tannins unless these various polyphenols are removed from or metabolized in the rumen. This latter seems to be feasible in the case of both the hydrolysable and condensed tannins for, according to Williams [43], tannic acid or albumin tannate administered to animals, including rabbits, results in the excretion of gallic acid whilst protocatechuic acid is said to be a normal constituent of the urine of herbivorous animals and is thought to be derived from the diet and especially from tannins, although it is also possible that it may be derived from non-tanning polyphenols which could serve as structural units for condensed tannins. In addition Beijerinck [3] reported that, when air was excluded, *B. aerogenes*, an organism which is normally injested with fodder, decomposed quinic acid in the presence of mineral salts giving carbon dioxide and propionic acid.

The combination of tannins with fibrous proteins is considered to be influenced to a large extent by the spatial arrangement of the protein molecules which is affected by the method of preparation of the hide for tanning, *e.g.* liming which removes interfibrillary proteins and cementing substances thereby making it easier for tannins and enzymes to penetrate the hide, and the characteristics, *e.g.* size range, of the tannin molecules. Since the molecules of the tannin—stripping agents are small in size it seems unlikely that the tannin which is not removed by these agents remains fixed because the agents are unable to reach it; it seems more likely to be due to its attachment to particular locations on the protein molecules. The tannin which cannot be removed by stripping agents may therefore be expected to be

distributed in a more or less regular pattern throughout the fibrous protein and only limited parts of protein molecules would be exposed to the action of enzymes. Further breakdown of the protein molecules by enzymes would presumably require the prior removal of tannin which does not seem to be removed by stripping agents; in the case of the protein—tannin substrate being in an environment such as soil it would seem necessary to postulate the intervention of an enzyme system although the effect of repeated washing by rain water in conjunction with the effect of $CaCO_3$ is not known; in acid soils at least it would seem that any purely chemical stripping of tannin must be an extremely slow process. Removal of fixed tannin by enzyme action would seem to require either piecemeal removal of phenolic nuclei or larger fractions of the tannin molecule or removal of complete tannin molecules by rupture of protein—tannin bonds; the latter seems rather unlikely for if the protein molecules themselves are inaccessible to enzymes due to the presence of tannins then the protein—tannin bonds may be expected to be equally inaccessible. Although fibrous protein—tannin complex in the form of leather is usually characterized by considerable resistance to decomposition, piecemeal breakdown of accessible tannin molecules of the protein—tannin complex, especially when all but fixed tannin has been removed, may not be ruled out especially in the case of the hydrolysable tannins for tannases which breakdown these substances, at any rate in the free state, are well known. In the case of the condensed tannins there do not seem to be any records of enzyme systems which can break down this type of tannin, if this means that such enzymes are of infrequent occurrence or that they are only formed or only active over a limited range of conditions this may be part of the explanation of the apparently greater stability of the complexes they form with proteins, although the rupture of non-ester bonds in the breakdown of quercitin by *Pullularia fermentans* has recently been recorded by Hattori and Noguchi [16] and Somerfield [35] concluded that some fungi, including *Penicillium solitum*, readily decompose (+)-catechin giving non-aromatic compounds. The participation of enzymes in the breakdown of leather seems to be indicated by the observations of Colin-Russ [6] under conditions where the action of purely chemical detanning agents was unlikely.

Piecemeal removal of the tannin molecules accessible to enzyme action of a fibrous protein—tannin system could result in protein molecules becoming accessible to enzyme action and by combined action of the tannin and protein-decomposing enzymes the protein—tannin complex would be decomposed.

In the case of globular proteins the orderly arrangement of the protein molecules found in the fibrous proteins is absent and, in terms of current ideas on the nature of the interactions between polyphenolic substances and proteins, the properties of such proteins in solution as far as combination with polyphenols is concerned will be governed to some extent by the concentration of the protein in the solution. In dilute solutions there will be little spatial restraint and all reactive groups will be accessible. Cross linkages by tannin molecules will become more likely as the concentration of the

protein solution increases. About the time of leaf fall the moisture content of the leaf will tend to decrease in many cases and the concentrations of the solutions of globular proteins may be expected to increase thereby increasing the opportunities for crosslinking by tannin molecules .The disorderly distribution of globular protein molecules in solution may also mean that tannin molecules which have undergone oxidative polymerization and which, as a result, appear to lose their ability to tan fibrous proteins may still be able to function as crosslinking tannins as far as globular proteins are concerned; this seems to be supported by the observation that gelatin—tannin (mimosa) complexes contain greater amounts of fixed tannin than the maximum amount of the same tannin found to combine with hide powder (Gustavson[13]).

It has been mentioned earlier that some of the tannin of the globular protein—tannin complexes is not removed by aqueous acetone and it may therefore be expected that the protein of such complexes will show resistance to decomposition by enzymes and if a pattern similar to that occuring in the case of fibrous protein—tannin complexes is followed, the degree of resistance will depend on the kind of tannin involved.

The differing resistance to decomposition of the proteins of the casein—leaf extract complexes, as indicated by their relative nitrogen-supplying capacity for the growth of birch seedlings, follows the pattern which would be expected, from the observations on the breakdown of the protein of fibrous protein—tannin substrates by enzymes, if Calluna leaves contain condensed tannins and Circaea and Chamaenerion leaves contain hydrolysable tannins, as the reactions of their extracts suggest may be the case. The differential resistance of these casein substrates to decomposition might have been even more marked if the birch seedlings had been grown with a root environment having a more acid reaction.

The suggestion that some of the important properties of the litter of different plant species, *e.g.* with regard to mor formation and nitrogen mobilization, are due to the properties of the leaf protein—tannin complexes they contain is supported by the results of the birch seedlings experiment, by animal feeding experiments and by experiments on the tryptic digestion of collagen tanned by various tannins. The influence of various environmental factors, physical and chemical, on the breakdown of such protein—tannin complexes, and because of the different kinds of tannin and polyphenol occurring in the leaves of different species there is the potentiality for the formation of many different protein—tannin complexes of differing properties in the leaves of different plant species, by biological systems requires to be studied if the role

of these complexes in soil processes, which are often delicately balanced, and plant and animal nutrition is to be understood more completely and especially if control of raw-humus formation is to be attempted.

Leaf protein—polyphenol complexes in relation to control of raw-humus formation, mobilization of litter nitrogen and the digestibility of fodder may be considered from another point of view. In addition to variations in the polyphenol content of leaves which it is suggested occur due to such factors as the stage of development of the leaf, intensity of illumination and supply of mineral nutrients to the plant, there appears to be evidence that the production of polyphenols in plants is influenced by their genetic constitution. It may therefore be profitable to enquire whether individuals, especially in the case of species whose litter gives rise ro raw humus, whose leaves do not give rise to leaf protein—polyphenol complexes, because the plants lack the capacity to produce tannins or their precursors, may be selected or produced as mutants. In addition to the possibility that such individuals might be more attractive to herbivores they might also have a greater susceptibility to pathogenic micro-organisms.

## SUMMARY

The nitrogen absorbed by birch seedlings grown in sand culture has been used as a measure of the resistance to decomposition of complexes formed in vitro by interaction between protein and water-extractable leaf constituents of *Calluna vulgaris*, *Chamaenerion angustifolium* and *Circaea lutetiana*. The resistance to decomposition of these complexes measured in this way is *Calluna vulgaris* > *Chamaenerion angustifolium* > *Circaea lutetiana*. This result seems to be in agreement with the soil-forming and field characteristics of these species and especially the raw humus-forming tendencies of *Calluna vulgaris*.

The nitrogen released, as measured by the nitrogen absorbed by birch seedlings, from *Calluna* raw humus (H-layer material) and the model protein—*Calluna* leaf extractives complex is very nearly proportional to the widely different amounts of total nitrogen initially added to the root environment thereby suggesting that the organic nitrogen of the model complex and of the *Calluna* raw humus have similar properties.

The amount of nitrogen absorbed by the birch seedlings when unaltered protein was added to the sand culture was less than when protein—*Circaea lutetiana* leaf extractives complex was added. This is ascribed to too rapid release and loss of nitrogen before it could be absorbed by the birch seedling

roots and suggests a role for some leaf protein complexes in the conservation of litter nitrogen. Some possible effects of protein complexes in relation to the digestion of fresh and dry leaf material of various plant species by animals are discussed.

Information from archeological observations and from investigations into the tanning of proteins is considered in relation to possible factors influencing the decomposition of protein complexes of litter and it seems probable that a less acid reaction, *e.g.* such as is brought about by the addition of adequate amounts of calcareous material, will assist the mobilization of the nitrogen of raw-humus-forming litter.

The contention that the protein-precipitating substances of leaves are of fundamental importance in soil processes, especially raw humus formation and the supply of nutrients for plant growth, is considered to be supported by experimental approaches from different directions.

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# CALCIUM-MAGNESIUM RATIOS IN THE ROOT MEDIUM AS RELATED TO MAGNESIUM UPTAKE BY CITRUS SEEDLINGS

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## INTRODUCTION

As previously reported [5], soil analyses in magnesium-deficient and in healthy Shamouti orange groves displayed exchangeable Ca/Mg ratios of 8.1 and 4.1 respectively. The assumption was made that calcium excess may impair magnesium uptake by citrus roots in the sandy soils of Israel's Mediterranean coast.

According to Jacob [4], however, there seems to be some controversy about the possibility of calcium retarding magnesium uptake by plants. Pfaff and Buchner [6] concluded that lime amendments to an acid soil may enhance magnesium uptake even more than neutral magnesium salts; on the other hand, liming a non-acid soil induces magnesium deficiency. The work presented here is a greenhouse pot experiment, carried out to verify the assumption that low Mg/Ca ratios in the root medium may be responsible for the low magnesium uptake by citrus trees.

## MATERIALS AND METHODS

### *A. Experimental procedure*

Six-week-old sweet-lime (*Citrus aurantifolia*) seedlings were grown in earthenware pots (three to a pot) filled with 3230 g soil. The cotyledons were excised before planting. The soils were adjusted beforehand to three exchangeable Mg/Ca ratios 0.33, 0.11, and 0.05 (Treatments 1, 2, and 3 respectively). The Ca + Mg/K ratio was constant in all treatments; the lowest Mg/K ratio used was 5.0. The pots were painted with an acid-resistant paint.

The root media were mixtures of calcium, magnesium, and potassium saturated sandy soils, with a cation-exchange capacity of 5.5 me per 100 g.

The mixtures were moistened to "field capacity" (determined in the pots), and incubated for two months.

Calcium-, magnesium- and hydrogen-saturated soils were prepared by rinsing the native soil with the respective chlorides; the excess was washed out with distilled water. Potassium-saturated soil was prepared by adding equivalent amounts of a potassium hydroxide solution to the hydrogen-saturated soil, and incubating for two weeks.

TABLE 1

The composition of the soil in the various treatments

| Treatment No. | Mg/Ca ratio in the soil | Grams of soil (per container) saturated with: | | |
|---|---|---|---|---|
| | | Ca | Mg | K |
| 1. | 0.33 | 2400 | 800 | 30 |
| 2. | 0.11 | 2880 | 320 | 30 |
| 3. | 0.05 | 3048 | 152 | 30 |
| 4. Pots | – | 3048 | – | 28.6 |
| 5. Pots | 0.05 | 2910 | 145 | 28.6 |
| 4. Each of three cups | – | – | 50.7 | 0.47 |
| 5. Each of three cups | 0.05 | 46 | 2.3 | 0.47 |

In two further treatments (4 and 5) the root systems of sweet-lime seedlings were split into two different containers: one a pot, as in the previous treatments, the other a plastic cup containing 50 g of soil. For every pot three such cups with double bottoms were prepared. Between the two bottoms an air space of 5 mm was left. This space prevented contact between the soils in cups and pots. A hole, big enough for the taproot of a sixth-month-old seedling, was bored through the double bottom. At planting time, the taproot of a seedling was inserted into the hole, leaving most of the root system in the cup. The cup was then filled with the appropriate soil mixture. Three such cups were inserted, for one quarter of their height, into the pot beneath the cup. The arrangement is shown in Plates 2 and 3. Treatment-4 pots contained a mixture of calcium- and potassium-saturated soils, while the cups contained a mixture of magnesium- and potassium-saturated soils. Treatment 5 served as a control; boths pots and cups were filled with a mixture of the above-mentioned calcium- magnesium- and potassium-saturated soils, in ratios identical with those in Treatment 3. The total Mg/Ca and Mg + Ca/K ratios in the root medium of Treatment 4 were also the same as in Treatments 3 and 5. The detailed composition of the soils in the different treatments is outlined in Table 1.

To all the pots were added: 21 me of $NO_3^-$, 3.5 me $H_2PO_4^-$ and 3.5 me $HSO_4^-$ adsorbed on Amberlite IR–4B, as well as 10 ml of a solution containing 7.25 mg $CuSO_2.5H$ O, 4.85 mg $MnSO_2.4H$ O, 0.211 mg $H_3BO_4$ and 7.25 mg $ZnSO_4.7H_2O$.

Each treatment was replicated five times. After 122 days leaf samples were taken from each pot for chemical analysis of the main cation contents.

### *B. Chemical analyses of plant material*

Leaves from the eighth node above the cotyledonary node were taken for analysis, on these leaves no deficiency symptoms were visible. After washing the leaves in demineralized water they were dried at 70°C and wet ashed [9]. In the ash solution magnesium was determined colorimetrically [1], calcium by difference between magnesium determined as mentioned, and the calcium plus magnesium determined with versenate [2]. Potassium was determined with a flame spectrophotometer (Beckman Model DU). From the split-root treatments, the different parts of the root system were also analyzed for calcium, magnesium and potassium contents by the same methods.

## EXPERIMENTAL RESULTS

### *A. Treatments 1–3*

During the experimental growth period definite magnesium-deficiency symptoms appeared on the lower leaves of the seedlings in Treatment 3 (see Plate 1). No such symptoms were visible on the leaves of seedlings from the other two treatments.

The leaf analysis showed a decrease in calcium and potassium contents and an increase in the magnesium content and in the Mg/Ca ratio as the Mg/Ca ratios in the soil were raised.

The coefficients for the correlation between calcium, potassium and magnesium contents in the leaves and the Mg/Ca ratio in the root medium were: —0.643 *, —0.545 * and 0.858 **, respectively.

TABLE 2

Calcium, magnesium and potassium contents and Mg/Ca ratios in the leaves of sweet-lime seedlings, grown in soils with various Mg/Ca ratios, and with their root system split or not split

| Treatment No. | Mg/Ca in the soil | Milliequivalents in 100 g leaf material | | | Mg/Ca in leaf material |
|---|---|---|---|---|---|
| | | Ca | Mg | K | |
| 1. | 0.33 | 199 | 21.5 | 23.6 | 0.108 |
| 2. | 0.11 | 227 | 15.6 | 27.6 | 0.070 |
| 3. | 0.05 | 254 | 9.53 | 29.5 | 0.037 |
| 4. (Roots split; Ca and Mg soils separated) | 0.05 | 204 | 17.7 | 29.8 | 0.087 |
| 5. (Roots split; Ca and Mg soils mixed) | 0.05 | 227 | 9.45 | 27.8 | 0.042 |
| L.S.D. at $P$ = 0.05 level | | 30.3 | 3.32 | 4.50 | 0.023 |
| = 0.01 ,, | | 40.8 | 4.38 | 6.35 | 0.031 |

* Significant at 0.05 level; ** significant at 0.01 level.

Plate 1. Sweet-lime seedlings grown for 122 days in soil with a Mg/Ca ratio of 0.05. Magnesium-deficiency symptoms are visible on the lower leaves.

Plate 2. Sweet-lime seedlings from Treatment 5. The two containers — plastic cups and pot — into which the roots were split, are shown. Magnesium-deficiency symptoms are visible on the lower leaves.

Plate 3. Sweet-lime seedlings from Treatment 4, at the end of the experiment. The lower part of the root system had been in the pot, the upper part is still in the plastic cup.

Of the three major cations, magnesium was most conspicuously influenced by the Mg/Ca ratio in the soil. Calcium and potassium contents were 22 and 20 per cent higher respectively, and magnesium contents 56 per cent lower in Treatment 3 than in Treatment 1. The detailed results are presented in Table 2.

*B. Split-root treatments (4 and 5)*:

1. Mineral contents of the leaves. Magnesium-deficiency symptoms appeared on the lower leaves of the control seedlings (Treatment 5) during the experimental growth period (Plate 2). Except for the splitting of the roots this treatment was identical with Treatment 3, in which deficiency symptoms had been observed. No deficiency symptoms were visible on the leaves of the seedlings from Treatment 4, in which the calcium-saturated soil had been separated from the magnesium-saturated soil (a seedling from this treatment is shown in Plate 3).

The chemical analyses for the major cation contents of the leaves showed no significant differences between Treatments 3 and 5 (Table 2), *i.e.* splitting of the root system did not significantly influence the cation uptake. Similarly, nog significant differences were found between calcium and potassium contents in leaves from the two split-root treatments (4 and 5). The analyses showed, however, a highly significant difference in the magnesium contents of the leaves from Treatments 4 and 5. As a result of the separation of magnesium- and calcium-saturated soils (Treatment 4), the magnesium contents were increased to 17.7 me per 100 g of dry material, as compared to 9.45 me per 100 g in leaves from the control seedlings (Treatment 5).

2. Cation contents in the roots. The calcium, magnesium and potassium contents in the roots of seedlings from the split-root treatments are presented in Table 3. It should be noted that highly significant differences were found between the two parts of the root systems in Treatment 5, even though these roots developed in the same medium. This difference may possible be explained by the fact that greater lignification occurred in the older portion of the seedlings' roots – the percentage of living tissue must have been much lower in the cups, containing the upper part of the tap root – than in the pots. Accordingly, comparisons between

TABLE 3

Calcium, magnesium and potassium contents in the two parts of the root systems of sweet-lime seedlings from split-root experiments (treatments 4 and 5)

| Treatment No. | Milliequivalents in 100 g of dry-root material | | | | | |
|---|---|---|---|---|---|---|
| | Ca | | Mg | | K | |
| | pots | cups | pots | cups | pots | cups |
| 4. Ca and Mg soils *separated* . . . . . | 33.9 | 15.0 | 21.4 | 33.2 | 13.0 | 13.7 |
| 5. Ca and Mg soils *mixed* . . . . . | 23.2 | 32.4 | 25.8 | 18.8 | 9.3 | 9.5 |
| L.S.D. at $P = 0.05$ level | 4.6 | | 4.1. | | 2.1 | |
| $= 0.01$ ,, | 6.3 | | 5.7 | | 2.5 | |

the two treatments can only be made by reference to roots from the same type of container. Such a comparison showed that the amounts of calcium and magnesium which accumulated in every part of the root systems were correlated to the composition of the soil in its immediate vicinity. The calcium content in the roots from the pots of Treatment 4, which contained no magnesium saturated soil, was significantly higher than that in the roots from the pots of Treatment 5, which contained a mixture of calcium- and magnesium-saturated soils. The opposite relations are encountered in roots from the cups of these same treatments: calcium contents of the roots from the controls were more than 100 per cent higher than in those from Treatment 4. Differences in magnesium contents of the roots from the pots of the two treatments, though significant, were small. These relatively small differences might be attributed to the ready mobility of magnesium ions, which allows it to be translocated from the roots in the cups of Treatment 4, to those in the pots. Magnesium contents in the roots from the cups of Treatment 4, containing only magnesium- and potassium-saturated soils, were by about 75 per cent higher than in the controls.

Potassium contents did not differ in the two parts of the root system of the same treatment. Potassium contents in the roots from Treatment 4, however, were higher than in those from Treatment 5. These data suggest that calcium and magnesium together impair potassium uptake more than equivalent amounts of each cation alone.

## DISCUSSION

The results from Treatments 1 to 3 indicate that magnesium uptake is impaired at low Mg/Ca ratios in the root medium. At a Mg/Ca ratio of 0.05 visual deficiency symptoms appeared, such symptoms being evidence of a quite severe deficiency [7]. The reason for the inadequate magnesium uptake may be either the low magnesium content of the substrate or an excess of calcium. The results of the split-root experiment seem to indicate that the problem is one of mutual cation interference, and not of their absolute contents. A magnesium content as low as 0.26 me in 100 g of soil was sufficient, in Treatment 4, for satisfactory growth without the development of magnesium deficiency. At the same magnesium level, but in immediate presence of excess calcium, severe magnesium deficiency appeared in Treatments 3 and 5.

It seems necessary to discuss the possibility that changing Mg/K ratios were responsible for the differences in magnesium uptake in the experiments presented here. The Mg/K ratios changed in these experiments concomitantly with the Mg/Ca ratios. According to Schachtschabel [10] and Homes [3], magnesium deficiency is to be anticipated, in various crops, when Mg/K ratios in the soil are lower than 3.2. Pratt *et al.*[8] showed this critical ratio for citrus to vary between 1.35 and 3.5, according to the depth of the soil layer sampled. In order to avoid potassium interference, 5.0 was chosen as the lowest Mg/K ratio in the present experiments. Furthermore, the relative potassium contents in the two parts of the root systems of Treatments 4 and 5 indicate, as pointed out previously, that calcium and magnesium in combination, compete with potassium at least as well, as each cation alone. The (Ca+Mg)/K ratio was the same in all the treatments.

In conclusion it appears, that the assumption made in the previous paper [5], *i.e.* that calcium excess may be responsible for the appearance of magnesium deficiency in citrus, under certain conditions, has been verified.

## SUMMARY

A pot experiment was carried out in the greenhouse, on sweet-lime (*Citrus aurantifolia*) seedlings grown in soils with three Mg/Ca ratios: 0.33, 0.11, and 0.05. Leaf analyses showed positive correlations between Mg/Ca ratios in

the medium and magnesium contents, and Mg/Ca ratios in the leaves. Using a split-root technique it has been shown that impaired magnesium uptake at the Mg/Ca ratio of 0.05 in the medium, is not due to its low magnesium contents, but rather to an excess of calcium.

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